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The Group of *Adiantum gracile* in Brazil and Environs

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ABSTRACT.—The *Adiantum gracile* group of Brazil and adjacent Bolivia is a natural group distinguishable from *A. tetraphyllum* and related species. We provide a key to the group and describe two new Brazilian species belonging to the group, *A. cinnamomeum* and *A. dawsonii*.

Adiantum is a large and widespread genus in the tropics, numbering approximately 200 species in the Neotropics, 65 to 70 of them growing in South America. Brazil has about 50 species, about 75% of the total known for the continent.

No recent monograph or revision exists for *Adiantum* in the Neotropics, nor has a comprehensive subgeneric classification of the genus ever been developed. In preparing keys for floristic works, most authors have used as principal characters lamina venation (anastomosing vs. free) and shape of the ultimate divisions (roughly rounded and not dimidiate vs. more or less oblong and usually dimidiate). Secondary characters include lamina division (simple, pinnate, bipinnate, or decompound), pinnule attachment (sessile vs. pedicellate), ultimate division articulation (articulate or not), and lamina apex shape (conform vs. gradually attenuate).

The species with bipinnate laminae, a conform terminal pinna, and oblong and usually dimidiate pinnules may be placed together conveniently as the *A. tetraphyllum* group. Delimited in this broad manner, the group includes about a third of the Neotropical species of *Adiantum*. Most, if not all, of the species of this group lack evenly and finely serrate sterile lamina margins that seem to predominate in other groups.

Within the *A. tetraphyllum* Willd. group *sensu lato,* four groups can be fairly readily distinguished, principally on the basis of their rachis and lamina indument. These groups are not yet fully characterized, nor are their constituent species known with certainty due to several names for which we have yet seen any specimens. Three of these groups are large and widespread in the Neotropics, but one small group is nearly restricted to Brazil: *A. gracile* Fée and two new species.

The species of the *A. gracile* group differ from the other three groups in having more pinna pairs (5–7), more pinnules on a pinna (35–40 pairs), and pinnules that often are approximate or nearly so, narrower (2–4 mm wide) but

relatively longer, usually at least slightly lobed, and in lacking an obvious midrib, although they could be obscurely dimidiate. In addition, the larger scales on the abaxial surface of the pinnules, when present, are several cells wide and long-ciliate throughout, much like those of the rachises and costae. These scales are in contrast to those of *A. tetraphyllum* and its other allies, which are narrowed and sometimes even uniseriate distally. In the *A. gracile* group, the rachises are conspicuously covered with cinnamomeous, linear-lanceolate scales with long-ciliate margins and base, at least when young; these scales are most persistent *in A. cinnamomeum.*

KEY TO THE SPECIES OF THE *ADIANTUM GRACILE* GROUP

1. Pinnules abaxially glaucous, the scales few or none, but rather inconspicuous, yellowish, sessile glands often present; pseudoindusia erose-ciliate at maturity, adaxially glabrous. Rhizome scales narrowly lanceate, dark to medium brown, sparingly denticulate. Medial pinnules ca. 2 times longer than wide, usually acute at the sterile apex, nearly round at the fertile apex . . . . . 3. *A. dawsonii*
1. Pinnules abaxially not glaucous or glandular, always sparsely scaly; pseudoindusia entire to erose, scarcely ciliate, adaxially glabrous or bearing toothed scales . . . . . 2.
2(1). Medial pinnules ca. 2 times longer than wide, obtuse to acute at the sterile apex, angular at the fertile apex; pseudoindusia glabrous; rhizome scales medium to dark brown, entire . . . . . 1. *A. gracile*
2(1). Medial pinnules ca. 3 times longer than wide, acuminate to acute at the always sterile apex; pseudoindusia bearing hairlike scales that are toothed at the base; rhizome scales dark brown to blackish, the margins sometimes slightly paler, always sparsely denticulate . . . . . 2. *A. cinnamomeum*

1. ***Adiantum gracile*** Fée, Gen. Fil. 116. 1852. Fig. 1A, B.

Plants terrestrial. Rhizomes stout, short-creeping, ca. 4–5 mm in diam., scaly, the scales somewhat shiny, essentially concolorous, medium to dark brown, narrowly lanceate, entire. Fronds monomorphic, 2-pinnate, (30)60–100 cm long, the laminae (7)20–30 cm wide; stipes approximate, black, adaxially sulcate, scaly, the scales appressed throughout or sometimes distally patent, concolorous, cinnamomeous, 2–3 mm long, narrowly lanceate with a filiform apex, strongly denticulate proximally; rachises similar to the stipes and their indument similar; pinnae oblong-lanceate, slightly decreasing at the base, tapering at the apex, (7)15–22 cm long, 1.5–2.2 cm wide, the lateral pinnae (3)7–10 pairs, patent, alternate, the terminal pinna conform, 1–1.5 times longer than the subtending pinnae 0.67–1 times as long as the medial pinnae; indument of the costae like that of the stipes and rachises; pinnules 27–43 pairs, ca. 2 times longer than wide, chartaceous, free-veined, without an evident midrib, the proximal pairs reduced, somewhat rounded or triangular, the medial pairs dimidiate, oblong to somewhat tapering, the acroscopic base truncate, the sterile apex obtuse to acute, the sterile margin irregularly and distantly serrate, except at the pinnule apex, the fertile apex angular, the distal pinnules ca. 1/2 as long as the medial pinnules, the adaxial surface of the pinnules glabrous or sparsely scaly near the sori, the veins slightly prominulous, the idioblasts

FIG. 1. Scanning electron micrographs of *Adiantum*. FIGS. 1A–B. Proximal and lateral/distal views of *A. gracile* spores (*Salino 393*, UEC). FIGS. 1C–D. Overall and distal views of *A. cinnamomeum* spores (*MacFarland et al. 283*, holotype US). FIGS. 1E–F. Suprabasal portion of a rachis scale and an pseudoindusial scale of *A. cinnamomeum* (*MacFarland et al. 283*, holotype US). FIGS. 1G–H. Proximal and distal views of *A. dawsonii* spores (*Dawson 14868*, holotype US).

inconspicuous or nearly so, the abaxial surface of the pinnules glabrescent, with patent, sparse, toothed scales ca. 0.5 mm long but otherwise similar to those of the stipes, glands absent, the veins slightly prominulous, the idioblasts conspicuous; *sori* oblong, up to 7 or 8 per pinnule; pseudoindusia glabrous, entire to erose; spores trilete, 25–30 μm in equatorial diameter, tetrahedral-globose without prolonged angles, the surface rugulate with a thin, fragmenting outer layer, and so sometimes appearing finely cristate.

TYPE: Brazil: *sin. loc., Claussen s.n.* (P, Morton photo 2596, photos GH, SP; possible isotype US1148302 [*Claussen 177* ex C]).

DISTRIBUTION: Endemic to central Brazil (Mato Grosso, Distrito Federal, and Goiás).

HABITAT: Slopes and stream banks in cerrado and campo vegetation, at ca. 50–850 m elevation.

SPECIMENS EXAMINED: **Brazil: Mato Grosso:** Sararé, RADAMBRASIL, Serra de Pedra (S. Aguapei), 59°25′W, 16°10′S, *Pires & Santos 16572* (UEC); Chapada dos Guimarães, Véu-da-Noiva, *Verardo 23668* (SP, UEC), same locality, *Salino 393,* 500 m (UEC), same locality, *Hatschbach 37628* (MBM, UC). **Goiás:** Ca. 1 km S of S. João de Aliança, ca. 850 m, *Irwin et al. 31972* (NY not seen, SP, US); Serra do Caiapó, 50 km S of Caiaponia, *Prance & Silva 59588* (GH, K, NY not seen, US). **Distrito Federal:** Brasília, *R. Windisch & Ghillány 253* (HB).

2. ***Adiantum cinnamomeum*** Lellinger & Prado, sp. nov. Figs. 1C–F, 2.

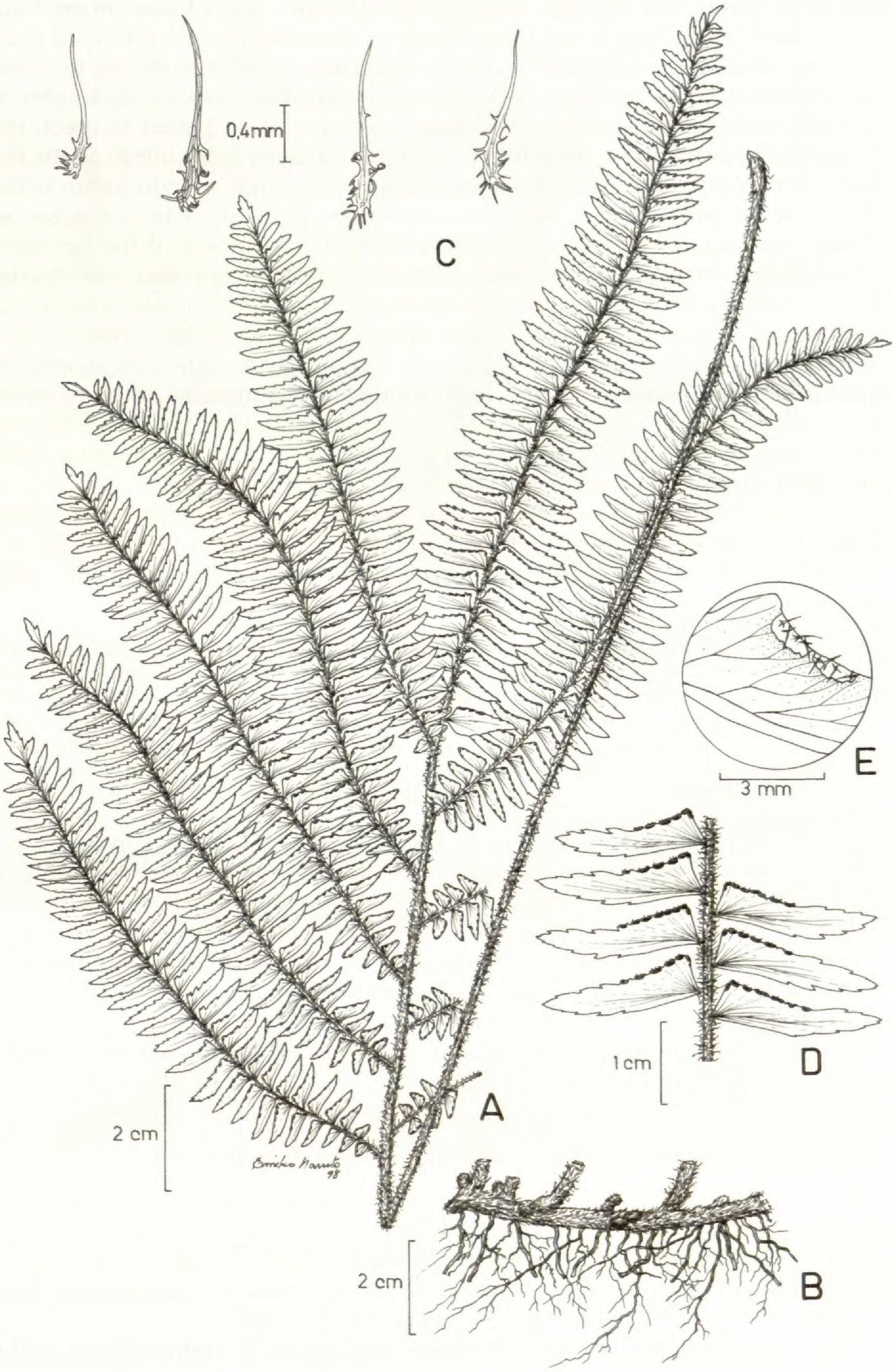
A specie *A. gracili* Fée paleis rhizomatis integris (vs. sparse denticulatis), pinnis 5–7 (vs. 8–10) jugatis patulis, pinnulis oblongis acuminatis, 3-plo (vs. 2-plo) longioribus quam latioribus differt.

TYPE: Brazil: Rondônia: 120 km SW of Pôrto Velho-Highway BR 364, 15 km W of Mibrasa, 9°10′ S, 63°07′W, 29 May 1982, *McFarland et al. 283* (holotype US photo SP; isotypes BM, GH).

Plants terrestrial. Rhizomes stout, short-creeping, ca. 3 mm in diam. excluding the protuberances, scaly, the scales shiny, slightly bicolorous, dark brown to blackish, narrowly lanceate, the margins lighter and sparsely denticulate. Fronds erect, monomorphic, 2-pinnate, 35–65 cm long, the laminae 20–25 cm wide; stipes ca. 5 mm distant, black, adaxially sulcate, scaly, the scales appressed throughout or distally patent, concolorous, cinnamomeous, 4–5 mm long, linear-lanceate with a filiform apex, sometimes long-ciliate at the base, medially copiously toothed-ciliate; rachises more densely covered by scales than the stipes, the indument similar to that of the stipes; pinnae oblong-lanceolate, slightly decreasing at the base, tapering at the apex, 8–19 cm long, 2.0–3.5 cm wide, the lateral pinnae 5–7 pairs, patent, alternate, the terminal pinnae conform, 1–1.25 times longer than the subtending pinnae, ca. 1.33 times longer and wider than the medial pinnae, the indument of the costae

→

FIG. 2. *Adiantum cinnamomeum.* FIG. 2A. Frond (*Steward et al. P20394,* US). FIG. 2B. Long-creeping rhizome (*MacFarland et al. 283,* US). FIG. 2C. Rachis scales (*MacFarland et al. 283,* US). FIGS. 2D–E. Abaxial surface of pinna and pseudoindusia showing scales (*Irwin et al. 10180,* US).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similar to that of the rachises; pinnules 35–40 pairs, ca. 3 times longer than wide, chartaceous, free-veined, without an evident midrib, the proximal pairs reduced, flabellate to subrhombic, the medial pairs dimidiate, oblong to somewhat tapering, 0.5–2 cm long, 2–4 mm wide, subarticulate (the dark color of the stalk terminating almost at the base of the pinnule), patent to erect, the acroscopic base truncate, roundish to straight, the apex subacute to acute, the sterile margins serrate to biserrate, except the basioscopic margin entire to the middle of the pinnule and dentate towards the apex, the distal pinnules ca. 1/4–1/5 as long as the medial pinnules, the adaxial surface of the pinnules glabrous, the veins not prominulous, the idioblasts conspicuous, the abaxial surface glabrescent, with sparse, cinnamomeous scales, the scales filiform, ca. 1.0–1.5 mm long, pectinate at the base, glands absent, the veins prominulous, the idioblasts inconspicuous; sori oblong, 7 or 8 per pinnule; pseudoindusia with filiform scales, bearing short basal processes, the margins entire to erose; spores trilete, 25–30 μm in equatorial diameter, tetrahedral-globose with prolonged angles, the surface sparsely rugulate with a thin, fragmenting outer layer, and so sometimes appearing finely and sparsely cristate.

DISTRIBUTION: North-central Brazil (Rondônia, Amazonas, Pará, Mato Grosso, Distrito Federal).

HABITAT: On clay soil, in open canopy and dense understory, at 50–1000 m elevation.

PARATYPES.—**BRAZIL: Rondônia:** Pôrto Velho to Cuiabá Highway, vicinity of Santa Bárbara, 15 km E of Km. 1117, *Prance & Ramos 7157* (NY, UC). **Amazonas:** Manaus-Caracaraí Highway, Km. 160, *Steward et al. P20394* (BM, MO, NY, US); Manaus-Caracaraí Highway, Km. 185, *Prance et al. 22691* (MO, NY, US); Rio Javari between Estirão do Equador and Rio Javarizinho, *Prance et al. 24046* (NY, UC); Manaus, Rio Araras, SIDERAMA, *Loueiro et al. s.n.* (GH); Manaus, *Pivetta 254* (HRCB); Barra, Prov. Rio Negro, *Spruce s.n.* (GH, US). **Pará:** Município de Oriximiná, Rio Trombetas, estrada da Mineração Santa Patrícia, ramal 22, *Cid et al. 1441* (NY, US); Rio Jamundá, Municipio de Faro, São Jorge, *Black & Ledroux 50-10761* (HB). Mato Grosso: Vila Bela da Santíssima Trindade, Faz. Cabixi, junto ao Rio Cabixi, 13°S, 60°10′W, ca. 12 km da divisa com Rondônia, *Prado & Salino 8* (HB, SPF, UEC). **Distrito Federal:** Parque Municipal do Gama, *Irwin & Soderstrom 5884* (NY, US); *Irwin et al. 10180* (GH, K, MO, NY, US); Brasília, Reserva Ecológica do IBGE, *Heringer et al. 3767* (MBM).

Adiantum cinnamomeum is distinguished by its long, narrow pinnules that are acuminate to acute at the apex, presumably because the sori are always lateral.

Adiantum cinnamomeum has been found only in Brazil, but may have a wider range in Amazonian South America.

3. ***Adiantum dawsonii*** Lellinger & Prado, sp. nov. Fig. 1G–H, 3.

A specie *A. gracili* Fée pinnulis adaxialiter glaucis, paleis paucis vel nullis, glandulis rotundis sessilibus luteolis differt.

TYPE: Brazil: Goiás: Southern Serra Dourada region, 20 km E of Formoso, 48°50′W, 13°45′S, on banks and margins of small stream running through hilly cerrado, 16 May 1956, *E. Y. Dawson 14868* (US, 2 sheets).

Plants terrestrial. Rhizomes stout, short-creeping, ca. 4–5 mm in diam., scaly,

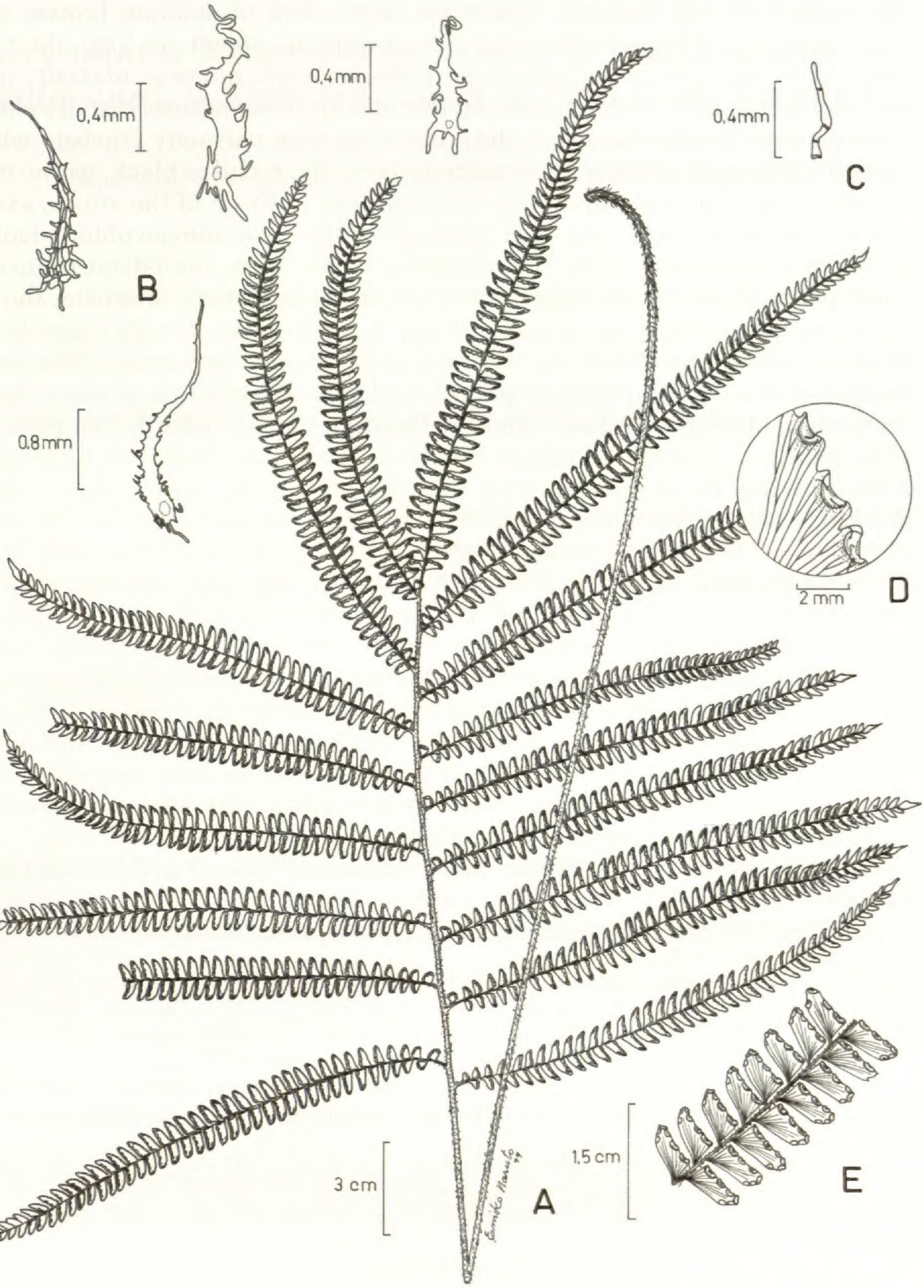

FIG. 3. *Adiantum dawsonii* (*Dawson 14868*, US2291503). FIG. 3A. Frond. FIG. 3B. Rachis scales. FIG. 3C. Pseudoindusial trichome. FIG. 3D. Pseudoindusia showing trichomes. FIG. 3E. Abaxial surface of pinnae.

the scales narrowly lanceate, somewhat shiny, dark to medium brown, sparingly denticulate. Fronds monomorphic, 2-pinnate, 40–90 cm long, the laminae (15)20–30(35) cm wide; stipes approximate, black, terete or adaxially planate, thinly scaly, the scales appressed throughout or sometimes distally patent, concolorous, cinnamomeous, 2–3(4) mm long, very narrowly lanceate with a long, filiform apex, weakly denticulate proximally; rachises black, terete, more densely scaly than the stipes, the scales similar to those of the stipes, except twice as wide, 3–6 mm long, and regularly denticulate; pinnae oblong-lanceate, slightly decreasing at the base, tapering at the apex, the lateral pinnae 7–8(10) pairs, (8)12–20 cm long, 1.25–1.75 cm wide, patent, alternate, the terminal pinna conform, up to 22 cm long, 2.25 cm wide, 1–1.25 times longer than the subtending pinnae and the medial pinnae, the indument of the costae similar to that of the rachises; pinnules (20)35–55 pairs, ca. 2 times longer than wide, chartaceous, free-veined without an evident midrib, the proximal pairs reduced, rounded-triangular, the medial pairs dimidiate, oblong to somewhat tapering, the acroscopic base truncate, the sterile apices usually acute, the fertile apices rounded, the sterile margins irregularly serrate, the distal pinnules ca. 1/3 as long as the medial pinnules, the adaxial surface of the pinnules glabrous or with a few hairlike scales, especially near the sori, the veins prominulous to only slightly so, the idioblasts usually conspicuous, the abaxial surface of the pinnules glaucous, the scales few or none, round, sessile, pale yellow glands often scattered over the surface, the veins slightly prominulous, the idioblasts inconspicuous; sori oblong, 3–5 per pinnule, one commonly apical; pseudoindusia glabrous or sparingly catenate-pilosulous, eroseciliate at maturity; spores trilete, 25–30 μm in equatorial diameter, tetrahedral-globose with prolonged angles, the surface rugulate with a thin, fragmenting outer layer, and so sometimes appearing finely cristate.

DISTRIBUTION: Central Brazil (Mato Grosso and Goiás) and Bolivia (Santa Cruz).

HABITAT: Slopes and stream banks at 500–1300 m elevation.

PARATYPES.—**BRAZIL: Mato Grosso:** Gorge of Véu de Noiva, Chapada dos Guimarães, *Prance et al. 19082* (NY not seen, R, UC, US), *Guarim Neto et al. 459* (HRCB); Serra da Chapada, Buriti, *Malme Regn. Exped. I 1712* (S not seen, US); *sin. loc., H. Smith s.n.* (US). **Goiás:** Southern Serra Dourada region, 20 km E of Formoso, 48°50′W, 13°45′S, *E. Y. Dawson 14869* (US); Chapada dos Veadeiros, 1 km E of Alto Paraíso on road to Nova Roma, ca. 1300 m, *W. R. Anderson 6305* (GH, HB, K, NY not seen, US); Rio Areias, *Glaziou 22636* (NY, P not seen Morton photo 2589a, fragm US); *sin loc., Glaziou 15726* (UC, US).

BOLIVIA: Santa Cruz: Velasco, Parque Nacional Noel Kempff M., Campamento Las Gamas, 14°48′00″S, 60°23′00″W, 900 m elev., *Arroyo & Keil 200* (MO).

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Dryopteris ×correllii hyb. nov. (*D. carthusiana* × *goldiana*), a Rare Woodfern Hybrid from Vermont

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ABSTRACT.—A hybrid woodfern, *Dryopteris carthusiana ×goldiana*, is described and named from a specimen (at DUKE) collected in Monkton, Vermont in 1937. This is the first reliable report of this hybrid. The report is based on morphology of the specimen; no living plants have been found for cytological study. Previous reports of this hybrid combination are excluded.

Interspecific hybrids are of special interest in the study of the woodfern genus *Dryopteris* Adans.; more than 150 are known worldwide (Knobloch), including some 25 in North America (Montgomery, 1982). Some of these, although apparently sterile (not forming viable spores), are surprisingly common, including *D. carthusiana* (Villars) H. P. Fuchs × *intermedia* (L.) A. Gray (= D. × *triploidea* Wherry) and *D. cristata* (L.) A. Gray × *intermedia* (= *D. ×boottii* (Tuckerm.) Underw.). Many potential hybrid combinations, however, have not been reliably reported, including the one reported here, the hybrid of *D. carthusiana* and *D. goldiana* (Hook.) A. Gray (but see Montgomery [1982] for a preliminary notice of this collection).

The plant shown in Figure 1 is the only known collection of this hybrid. It was collected by Donovan S. and Helen B. Correll in Monkton, Addison Co., Vermont, an area of abundant *Dryopteris* populations that include both parents. In addition to the abortive spores, it is distinguished by a number of characters that, to field workers familiar with the participating parents, are readily evident and not matched by any other hybrid combination. Specifically, it differs from *D. carthusiana* in the less fine (bipinnate vs. bipinnate-pinnatifid) cutting of the lamina, blunter (less spinulose) toothing, blunter pinnules, and dark (vs. pale) scales at the base of the stipe. From *D. goldiana* it differs in its finer cutting, triangular lower pinnae, and stalked proximal pinnules. Sometimes, *D. goldiana* itself may be quite spinulose, e.g. *Winslow 10102* (Vermont: Westmore: Mt. Pisgah, 19 July 1910, NEBC). However, these uncommon forms do not have triangular basal pinnae, stalked pinnules, or other characters evident in the hybrid. A closely related hybrid, *D. goldiana* × *intermedia*, is well distinguished from true *D. carthusiana* × *goldiana* (Evans and Wagner, 1964). Most significantly, that hybrid has abundantly glandular indusia and glands along the rachis; these are clear markers of *D. intermedia* parentage (Montgomery, 1982). In contrast, this hybrid is eglandular

[1] Corresponding author.

FIGURE 1. Correll's hybrid woodfern, *Dryopteris* ×*correllii* Wagner (*D. carthusiana* × *goldiana*), from Monkton, Addison Co., Vermont. This is the only known specimen.

throughout. Also similar in overall aspect is *D. clintoniana* × *goldiana* (Dowell, 1908), but that hybrid has adnate pinnules, vs. the stalked pinnules of *D. carthusiana* × *goldiana.*

In keeping with an American tradition of naming woodfern hybrids for prominent botanists, this singular hybrid is here named for its discoverer, the late Donovan S. Correll (1908–1983).

Dryopteris ×correllii Wagner, *hyb. nov.*—TYPE: Vermont: Addison Co: Monkton; In bog near Monkton, 29 July 1937, *D. S. & H. B. Correll 7799A* (DUKE).

Planta hybrida e *Dryopteride carthusiana* et *D. goldiana* exorta, inter ambobus aspectu, a parentibus sporis abortivis differt. Frons usque ad 90 cm longinquitate (stipes 35 cm, lamina 55 cm); infimus stipitis vestitus squamis oblongi-linearibus, refulgentibus, castaneis, lamina subdeltoidea, bipinnata; pinnae proximae stipitatae; glandes laminis et indusiis desunt.

Stout terrestrial fern arising from hybrid parentage (*Dryopteris carthusiana* × *D. goldiana*) and of intermediate aspect. Spores abortive. Overall frond to 90 cm long (stipe 35 cm, lamina 55 cm). Basal stipe scales large, dark shiny brown. Lamina subdeltoid, bipinnate. Proximal pinnules conspicuously stalked, triangular; basal basiscopic pinnules longer than next most basiscopic pinnules; pinnules blunt. Laminar and indusial glands absent.

Only the specimen at DUKE is preserved. Duplicates may have been given to Dr. Henry Oosting for exchange, but to date we have not located any.

Wagner has discussed elsewhere various collections that had been suspected of being this hybrid. *Dryopteris ×poyseri* Wherry from Swarthmore, Delaware Co., Pennsylvania, was the first plant reported as this hybrid (Benedict, 1909), but studies showed it to be a bizarre form of *D. clintoniana* (Wagner and Wagner, 1982, Figs. 1 and 2), as originally thought by its discoverer (Clute 1908).

More recently, a plant considered to be *D. carthusiana* × *goldiana* has been reported from Clarendon, Rutland Co., Vermont (Thorne and Thorne 1989). However, examination of this specimen (*F. H. & E. Thorne s.n.,* 14 July 1981) shows it to be an odd frond of *D. carthusiana.* The specimen is barren of spores, sporangia, or indusia, and lacks any scales; it is more spinulose than the specimen from Monkton. Furthermore, it shows anomalous developmental characters such as forked pinnae and distally winged costae. Therefore, this report is also excluded. As far as we know at present, the Monkton specimen is unique. Why this hybrid does not occur more frequently is an enigma.

ACKNOWLEDGEMENTS

Wagner thanks Dr. Robert Wilbur, Curator of DUKE, for the loan of this specimen. Gilman thanks Dr. Florence S. Wagner, Dr. James D. Montgomery and anonymous reviewers for comments on the manuscript, and Libby Thorne for loan of the Clarendon specimen, which will be deposited at VT.

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The Relictual Fern Genus *Loxsomopsis*

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ABSTRACT.—We conducted a systematic revision of the genus *Loxsomopsis* H. Christ (Loxomataceae) based on 99 herbarium sheets representing about 52 separate gatherings. Many morphological characters varied considerably, but as much of this variation was found within individual collections it was of little taxonomic relevance. Characters that varied between specimens but remained roughly constant within individual gatherings included the pubescence of lamina, petiole, and sorus, hair size and coloration, presence or absence of glaucous layers on the lamina surface, and spore surface structure. However, we found no correlation in the occurrence of two or more of these characters and were unable to morphologically define discrete species within the genus. Thus, we consider *Loxsomopsis* to include one variable species, *L. pearcei* (Baker) Maxon. The morphological variation is probably the result of the distribution of the species in small, isolated populations in ephemeral early successional habitats, leading to continuous population extinction and establishment of new population based on few propagules. Notes on ecology and distribution, full taxonomy, and specimen citations are provided.

Loxsomopsis Christ is the Neotropical representative of the relictual and taxonomically isolated fern family Loxomataceae, whose other genus, *Loxoma,* is restricted to the North Island of New Zealand. *Loxsomopsis* is distinguished from *Loxoma* principally by its sporangia in which about two-thirds of the annulus cells are thickened (*vs.* only the apical cells thickened) and which open transversely (*vs.* longitudinally). After considerable debate as to the taxonomic relationships of the family (Tryon and Tryon, 1982; Kramer, 1990), it is now believed to be related to the tree fern alliance, along with Cyatheaceae, Dicksoniaceae, Lophosoriaceae, and Metaxyaceae on the basis of anatomical, morphological, and molecular characters (Markham and Given, 1979; Stevenson and Loconte, 1996; A.R. Smith, pers. comm.).

The genus *Loxsomopsis* was described by Christ (1904) based on *L. costaricensis* from Costa Rica, but the first species was actually described in 1891 by Baker as *Dicksonia pearcei* from Ecuador and was transferred to *Loxsomopsis* by Maxon in 1933. Two further species have been described, *L. lehmannii* from Ecuador by Hieronymus in 1904, and *L. notabilis* from Bolivia by Slosson in 1912. Because of the distinctness of the genus, there has been little debate about the generic delimitation and the placement of individual species. The delimitation of the species, however, is less clearcut and it has been suggested that only one species should be recognized (Tryon and Tryon, 1982; Tryon and Stolze, 1989). Overall, *Loxsomopsis* appears to be rare and/or localized in the field and is poorly represented in herbaria. A modern treatment for the genus is lacking. The present study is an outcome of a course on pteridophyte taxonomy and systematics and is based on a morphological anal-

ysis of 99 herbarium sheets of *Loxsomopsis* representing about 52 separate gatherings from 15 herbaria.

Morphological Characters

Stems.—Stems are terrestrial, long-creeping, dichtomously branched, dark brown, 1.5–5 mm in diameter, and with internodes 1–7 cm long. Scattered, dark, fibrous roots are produced from all sides. A physical relationship of the roots to the leaf bases was not noticed. Stems are densely pubescent with stiff bristles (defined as in Lellinger, 1985, by having pluriseriate bases and an uniseriate apex) that are dark brown, 0.8–2.4 mm long, basally pluriseriate with about 8–16 cells, and with an uniseriate apex consisting of 3–25 cells (Fig. 1A). The entire variation of bristle length and cell number can be found on individual stems. Presumably depending on the growth location, bristles can be erect or appressed, with both conditions as well as intermediates present on the same stem. The stems are solenostelic (Ogura, 1972). Root cortex anatomy corresponds to the *Osmunda*-type (Schneider, 1996), with a hypodermis of sclereids and inner cortical layers formed by relatively thin-walled cells.

Petioles, rhachises and costae.—Petioles are adaxially convex to flattened-sulcate and 0.6–4 mm in diameter. Coloration varies from dark blackish or reddish brown to pale olivaceous brown, with the proximal portion generally being darker. The proximal 1–2 cm of the petioles are generally densely covered in bristles resembling those of the stem. Scattered bristles are also found further along the petioles, especially in the groove. Frequently, petiole bristles have been abraded, leaving raised multicellular scars. Petiole length comprises about 45% to 65% of the entire leaf length. A single, gutter-shaped vascular bundle arrangement is present in the petioles (Kramer, 1990).

Rhachises are similar to the petiole, but tend to be somewhat more strongly sulcate, the groove more pronounced in dry than in fresh material. Rhachis coloration is generally paler than that of the petiole and is markedly darker on the adaxial than on the abaxial side. The costae, when dry, are rounded to slightly sulcate with a brown base and pale yellowish medial and distal portions. Scattered hairs (defined as in Lellinger, 1985, as being uniseriate throughout), resembling those of the lamina, occur occasionally on the costae.

Laminae.—Leaves are monomorphic, catadromous, subcoriaceous, circinnate in the bud, and with lamina length varying from 0.2 to 1.5 m. Leaf lengths of 5 m reported by Tryon and Tryon (1982) and Tryon and Stolze (1989) were not evident in herbarium material available to us nor observed in the field (M. Kessler, pers. obs., M. Lehnert, pers. obs.). Leaf dissection varies from 1-pinnate-pinnatifid on small leaves to bipinnate-pinnatifid on the largest leaves (Fig. 2). The pinnae are (sub)sessile, well-spaced, subopposite, asymmetric, with the proximal pinnae generally being the longest, reaching about 50% of the lamina length resulting in a narrowly to broadly deltate lamina outline. Secondary pinnae and segments are obliquely ascending, decurrent, with crenate to entire margins, and with the ultimate segments deltate-lanceolate. On

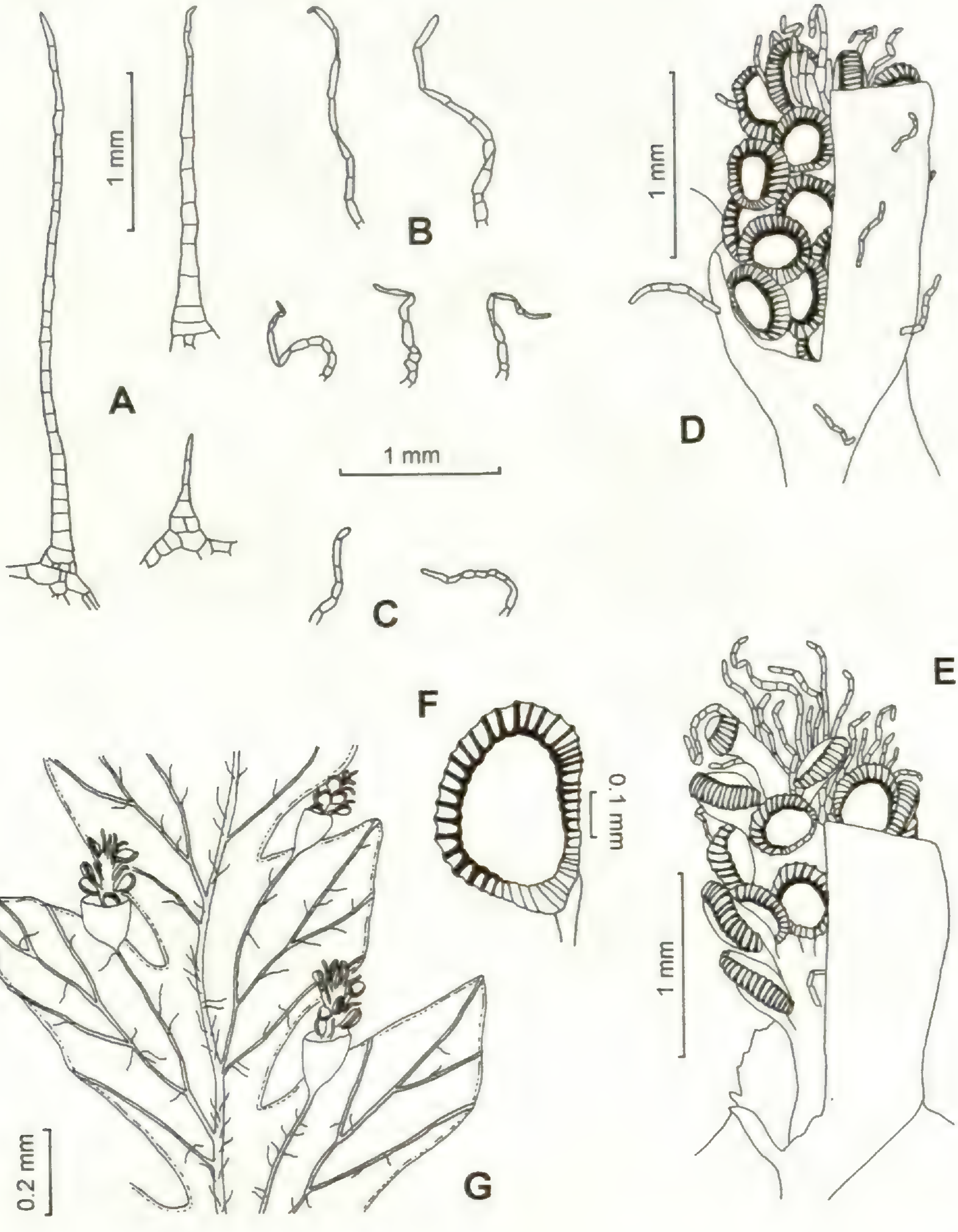

FIG. 1. Morphological details of *Loxsomopsis pearcei*. A) Stem bristles, showing variability in size and cell number, *Moran 3057*; B) Variability of lamina surface hairs, *Moran 3057*; C) Hairs from indusium, *Moran 3057*; D) Young sorus with short receptacle and sporangial stalks; left side of hairy indusium removed, *Øllgaard 74387*; E) Ripe sorus with lengthened receptacle and sporangial stalks; left side of glabrous indusium removed, *Lent 738*; F) Sporangium, showing the subvertical annulus with about ¾ of the cells indurated, *Øllgaard 74387*; G) Detail of pinna, showing free, forked-subpinnate venation, sorus location on vein ending at lamina margin, and pubescence on adaxial side, *Goldgewicht 86369*.

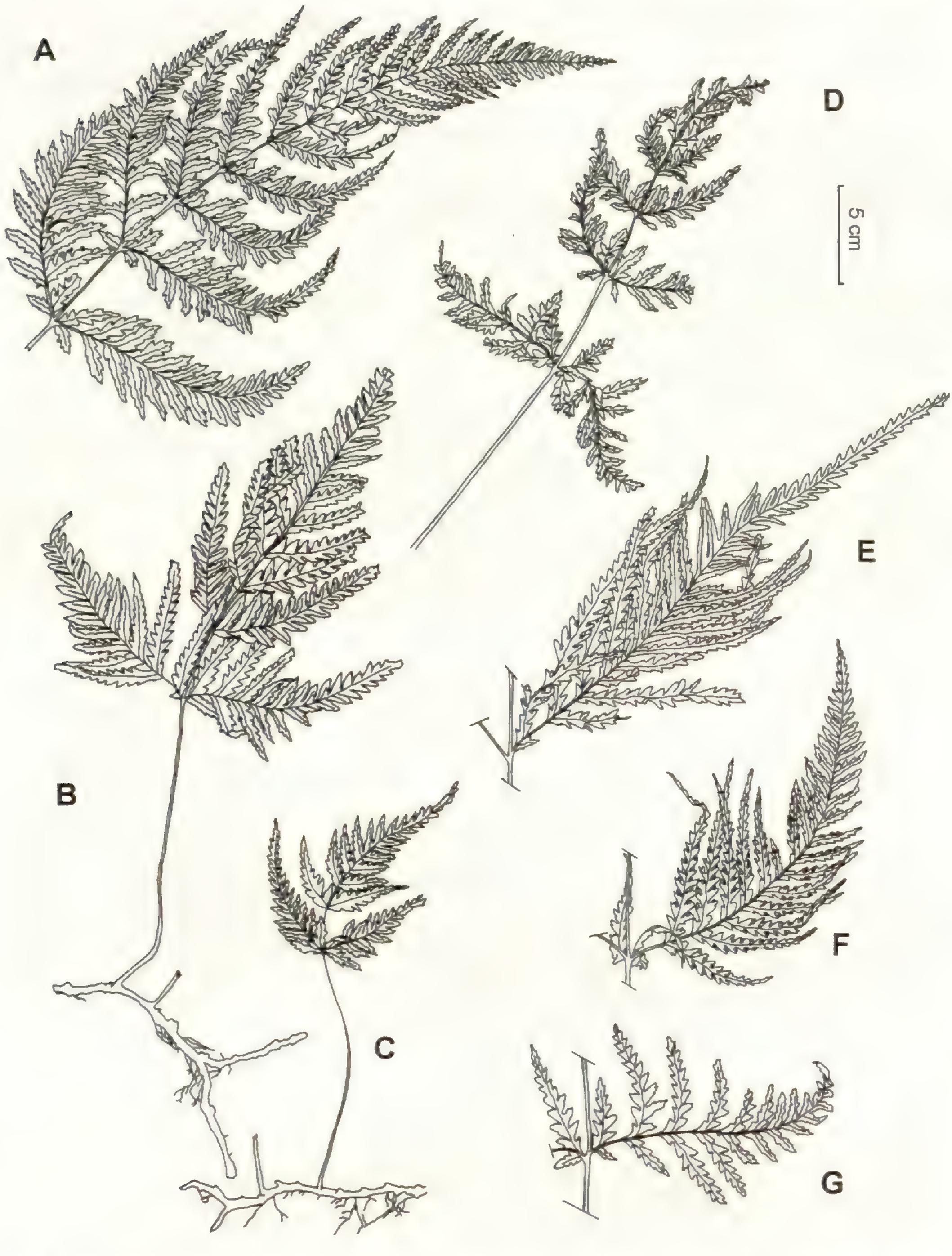

FIG. 2. Variability of lamina size, dissection, and shape. A) Distal portion of a large leaf, shown without the three proximal pinna pairs, *Grayum 7212*; B) Fully-developed, fertile, mid-sized leaf, *Prieto 259*; C) Fully developed, sterile, very small leaf, *Øllgaard 90634B*; D) Mid-sized leaf with notably differing segment shape, *Lehmann 5715*; E–G) Proximal pinnae showing differences in degree of dissection, segment orientation, and shape, *Lehmann 5059*, *Goldgewicht 86369*, *Goldgewicht 863*.

fertile leaves, acroscopic first- and second-order segments are larger than their basiscopic counterparts, whereas on sterile leaves they can occasionally be of similar size. Degree of lamina dissection, shape of pinnae and pinnules, and orientation of the pinnae vary considerably between leaves and individuals (Fig. 2). Smaller leaves tend to have longer, narrower, ascending pinnae, whereas larger leaves tend to have broader, more spreading pinnae. During lamina development, segments tend to be more rounded than on mature leaves. Veins are free, forked-subpinnate and catadromously branched (Fig. 1).

Laminae of *Loxsomopsis* can be glabrous to densely pubescent abaxially with uniseriate, up to 2 mm long, light to brown, tortuous, septate hairs (Fig. 1). The frequency and coloration of this vestiture varies geographically, with Costa Rican specimens characterized by long, pale hairs whereas specimens from some Ecuadorean locations have dark brown hairs. Bolivian specimens tend to have a glaucous abaxial lamina surface which varies among and within collections (Tryon and Stolze, 1989). Field observations have shown that older laminae are more glaucous than younger ones (M. Lehnert, pers. obs.). Adaxially the laminae are always glabrous.

Sori.—Sori of *Loxsomopsis* are located marginally on veins and are directed downward from the lamina surface. Generally, a single sorus is located on the acroscopic side of each terminal pinnule or ultimate segment, although ocassionally a second sorus is found basiscopically. Sori are narrowly cyathiform to urceolate and have a laterally free and protruding indusium with an entire rim. Superficially, they resemble sori of the Hymenophyllaceae. Indusia are glabrous to sparsely pubescent with hairs (Fig. 1D) resembling those of the laminae (Fig. 1C).

Sporangia and paraphyses are borne on a columnar receptacle whose basal portion elongates during sorus maturation, eventually measuring as much as 50% of the entire indusium length (Fig. 1D, E). As a result, young sori have fairly narrow indusia which conceal most of the sporangia, whereas in mature sori the majority of sporangia protrude beyond the more open indusium. Ripe sori are about 1 mm wide and 1.75–2.75 mm long. Paraphyses are uniseriate, unbranched, and without thickened cells, 0.5–0.8 mm long, and consist of 5–10 cells which are about 2–3 times longer than wide.

Sporangia.—The number of sporangia per sorus varies from (10)15 to 30(50), with much of this variation found on single leaves. Sporangia are borne on short, ca. 6-rowed stalks that lengthen somewhat during sorus maturation. Sporangia are more or less pyriform (Fig. 1F), about 350 μm wide and 450 μm long, with very little variation in size among and between collections. The annulus is complete, almost vertical, bypasses the stalk, and consists of 30 to 55 cells. Annular cells are indurated, except for a group of laterobasal cells which are smaller and have thinner cell walls. These thin-walled cells make up about 1/4 of the annulus (Fig. 1F).

Spores.—Spores of *Loxsomopsis* are trilete, tetrahedal-globose with prominent angles, with the laesure 1/2 to nearly equal the spore radius, and lack chlorophyll. Spores are 41–62 μm in diameter, with much of this variation found in single sori, e.g., 41–60 μm (n = 20, mean = 54 μm) measured in

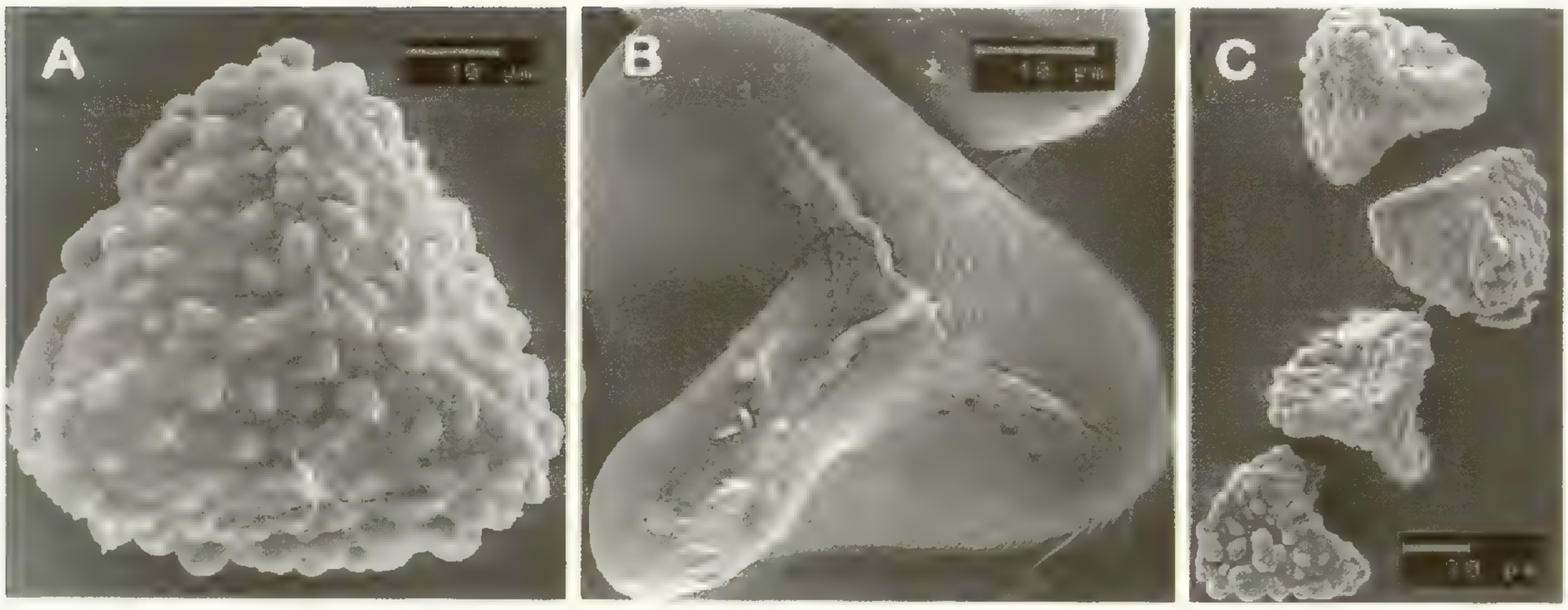

FIG. 3. Spores of *Loxsomopsis pearcei*. A) Verrucate spore with irregular granular deposit, *Goldgewicht 86369*; B) Slightly pitted spore, *Moran 3051*; C) Variability of surface structuring in verrucate spores, *Øllgaard 9479*.

Goldgewicht 86369. In three cases (*Bües 2119, Harling 24597, Lent 738*) the number of spores per sporangium was determined to be 128. Spore surface is slightly pitted (Fig. 3B) to strongly verrucose (nomenclature after Lellinger and Taylor, 1997) with distinct humps or longish, coalescent ridges (Fig. 3A; see also Tryon and Lugardon, 1991:213). These structures are formed by a two-layered perispore (Tryon and Lugardon, 1991). Spores derived from a single specimen are generally similar, but there is some variation in the degree of coalescence of the tubercules (Fig. 3C). The spores are often, but not always, covered with an irregular granular deposit (Fig. 3A).

Gametophytes.—Gametophytes of *Loxsomopsis* were described by Stokey and Atkinson (1956). They are epigeal, chlorophyllous, obcordate to somewhat elongate, centrally thickened, with thin margins, and bear long, multicellular hairs. Archegonia are borne on the lower surface of the thickened portion, especially apically, with the several- to many-celled antheridia located on the lower surface, and less often on the upper surface.

Chromosome number.—*Loxsomopsis* has been reported as $n = 46$ from Cerro Vueltas, Costa Rica, by Wagner (1980).

TAXONOMIC CONSIDERATIONS

Numerous morphological characters exhibited considerable variation. Many of these characters varied as much within as between collections and were thus of little taxonomic relevance. Such characters include overall leaf size, stem and petiole diameters, relative petiole length, degree and shape of lamina dissection, size of the stem and petiole bristles, the number of annulus cells, the number of sporangia per sorus, and spore size.

Characters that varied between specimens but remained roughly constant within individual gatherings included the pubescence of lamina, petiole, and sorus, hair size and coloration, presence or absence of glaucous layers on the lamina surface, and spore surface structure. However, we were unable to find

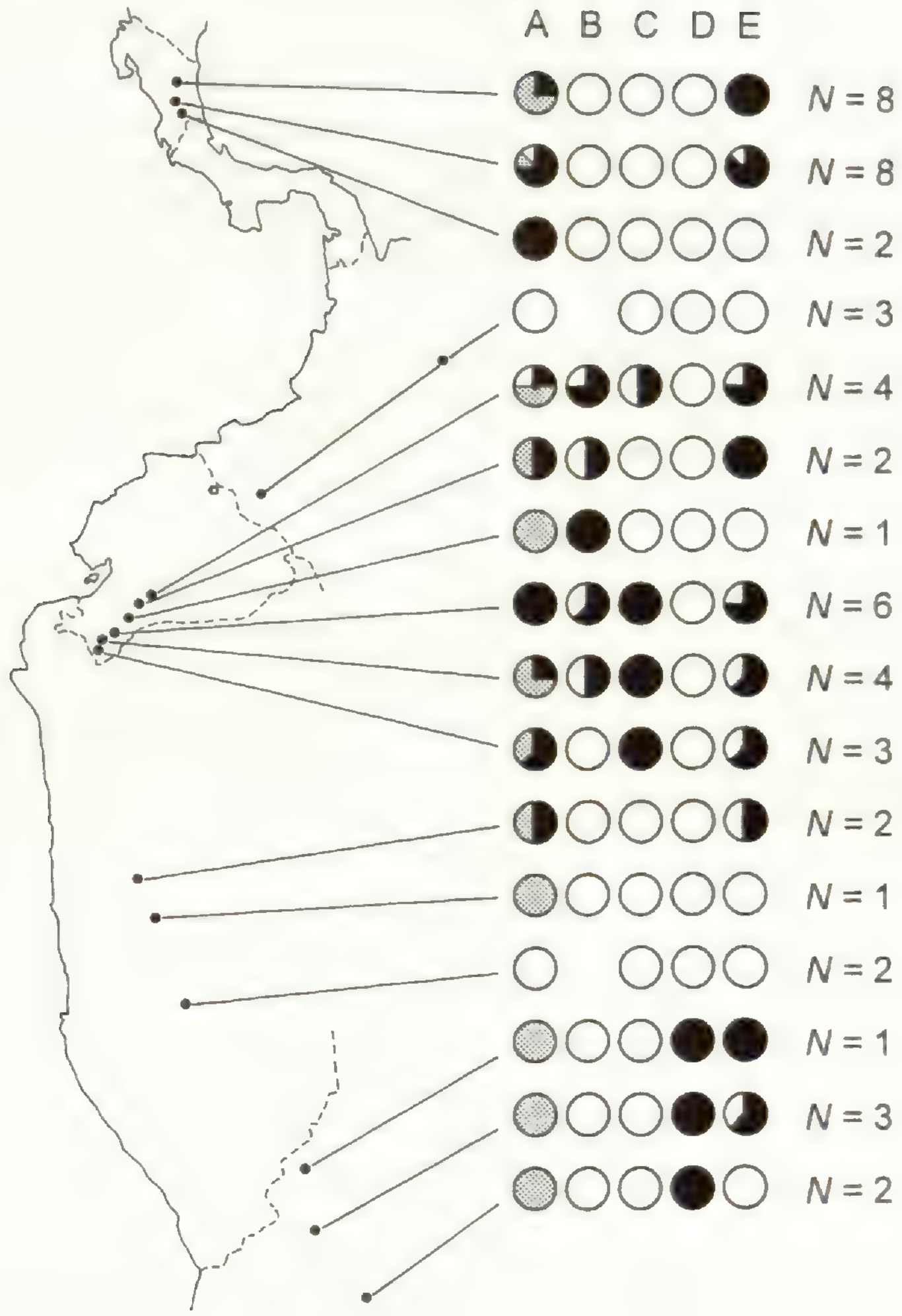

FIG. 4. Geographical distribution of several variable morphological characters among 52 gatherings of *Loxsomopsis pearcei* from 21 localities, indicating the number of gatherings per site (*N*). Characters and character states: A) Lamina pubescence: white: glabrous, gray: slight, black: dense (petiole pubescence followed the same pattern and is thus not shown as an independent character). B) Lamina hair coloration: white: pale, black: brown. C) Indusium pubesence: white: glabrous, black: hairy. D) Glaucous lamina surface: white: absent, black: present. E) Spore surface: white: smooth to slightly pitted, black: verrucose. Due to sterile or incomplete material, not every gathering has been considered for each character. The open circle shows the locality of a specimen not studied by us (*Palacios and Clark 12463* (MO)).

a correlation among these independent characters. There were some noticeable geographic trends, but exceptions were also found (Fig. 4). For example, Costa Rican specimens are characterized by rather long, pale and frequently abundant lamina hairs, but one specimen (*Evans 2660A*) was completely glabrous. Furthermore, the vestiture of some Ecuadorian and Peruvian collections re-

sembled that of the Costa Rican specimens. Dark brown hairs were restricted to Ecuadorian populations, but occurred in only about 60% of the specimens examined. Bolivian specimens were all characterized by a glaucous abaxial lamina surface. Pubescent indusia were found only in Ecuador, but not among all specimens from the region. The conspicuous differences in spore surface structure were specimen-specific and not related to the developmental stage of the spores, as has been reported, e.g., among Polypodiaceae (van Uffelen, 1997). None of these geographical trends were correlated with variation in other independent characters. Overall, the apparent independence of character variation precludes the delimitation of multiple taxa within *Loxsomopsis*. A comparable situation of a variable species which cannot convincingly be divided into discrete species is presented by *Plagiogyria semicordata* (Zhang and Nooteboom, 1998).

While cytological or molecular analyses are lacking, we found no evidence to suggest that different ploidy levels are represented within *Loxsomopsis*. Spore size varied as much within as between individuals and we found no individuals with consistently malformed or abortive spores that would indicate hybridization between plants of different ploidy levels.

We interpret the considerable morphological variation among different populations of *Loxsomopsis* to be a result of the localized and ephemeral nature of the growth sites of the species and its overall rarity. Since *Loxsomopsis* occurs mostly on disturbed sites such as landslides and roadsides, new populations are presumably regularly formed and then quickly lost as conditions are altered during vegetation succession. The result is a dynamic metapopulation structure in which new populations are frequently formed by one or few spores (Eriksson, 1996). Such a population structure would favor genetic drift and would explain the independent variation of morphological characters.

In summary, we have been unable to detect any character combinations that would permit the delimitation of different taxa within *Loxsomopsis*. While we cannot rule out the possibility that *Loxsomopsis* includes cryptospecies, we have found no evidence to support this assumption and consider the genus to include a single variable species, *Loxsomopsis pearcei*.

Geographic Distribution

Loxsomopsis is known from Costa Rica, Colombia, Ecuador, Peru, and Bolivia (Fig. 4). In Costa Rica, *Loxsomopsis* is confined to three separate populations, corresponding to the volcanoes Poas and Barva, and to the Cordillera de Talamanca. In Colombia, specimens have been obtained from the eastern Andean chain near the Venezuelan border and from both sides of the Andes close to the Ecuadorean boundary. While not yet known from Venezuela, the species may well occur there. In Ecuador, there is one specimen of *Loxsomopsis* from the northwestern Andean slope, but most collection come from the central and southern portion of the eastern Andean slope, where it has been collected along all major roads traversing the region, and presumably occurs

as one fairly large, nearly continuous population. The species appears to be absent from the botanically rather well-known northern portion of the eastern versant. In Peru, *Loxsomopsis* has been collected at three scattered localities in the central portion the country. Its absence from northern Peru, including several well-collected localities in the departments Amazonas and San Martin may well represent a real distributional gap. In contrast, the lack of collections from southern Peru may be a result of the low collecting activity in the region. In Bolivia, there are three known localities for *Loxsomopsis,* two of which, the La Paz-Coroico and the Cochabamba-Villa Tunari roads, correspond to the most intensively studied areas in the country. We suspect that *Loxsomopsis* occurs in intermediate areas as well.

Ecology

Loxsomopsis has been collected between about 1950 m and 2700 m in Costa Rica, and between 1800 m and 3600 m in the Andes. The majority of specimens have been gathered from roadside and forest edge habitats, but numerous specimens from Volcan Poás in Costa Rica have been found along the margin of the volcanic crater lake. Tryon and Tryon (1982) cite open habitats in ravines, on brushy slopes and open woodland as growth sites for *Loxsomopsis.* In the Andes, *Loxsomopsis* occurs on slopes disturbed by landslides and along roadsides, growing syntopically with aggressive colonizing ferns of the genera *Sticherus* (Gleicheniaceae) and *Hypolepis* (Dennstaedtiaceae) with which it forms dense tangles (León and Young, 1996, M. Kessler and M. Lehnert, pers. obs.). Leaves of *Loxsomopsis* are sometimes long with a pendent apical portion and lean on adjacent shrubby plants (Tryon and Tryon, 1982). *Loxsomopsis* has been collected on a wide variety of geological substrates, ranging from volcanic soils in Costa Rica to sandy soils and lutites in the Andes.

Generally, *Loxsomopsis* appears to be rather rare. In Bolivia, it has been recorded only four times in a total of about 300 vegetation plots (of which approximately 90 covered suitable early- to mid-successional vegetation) located in the right elevational belt within the distributional area of *Loxsomopsis* (M. Kessler, unpubl. data). In southeastern Ecuador, *Loxsomopsis* is locally common on roadsides (B. Øllgaard, pers. comm.).

Taxonomic Treatment

Loxsomopsis H. Christ, Bull. Herb. Boiss. II, 4:399. 1904.—Type: *Loxsomopsis costaricensis* H. Christ.

A monotypic genus, with the characteristics of its single species.

Loxsomopsis pearcei (Baker) Maxon, Proc. Biol. Soc. Wash. 46:105. 1933. *Dicksonia pearcei* Baker, Ann. Bot. (Oxford) 5: 197. 1891. *Dennstaedtia pearcei* (Baker) H. Christ, Index fil. 218. 1905.—Type: Ecuador, "Eastern Andes, 8000–9000ft." *Pearce 251* (Holotype, K!; Photo, US).

Loxsomopsis costaricensis H. Christ, Bull. Herb. Boiss. II, 4:399. 1904.—Type: Costa Rica, *Wercklé 279* (Holotype, P!).

Loxsomopsis lehmannii Hieron., Bot. Jahrb. Syst. 34:435. 1904.—Type: Ecuador, [Morona-Santiago,] prope Chinguinda, declivibus montium Cordillera Oriental de Sigsig, 1800–2500m, *Lehmann 5061* (Isotype, K!, US!). According to Tryon and Stolze (1989) holotype destroyed in LZ.

Loxsomopsis notabilis Slosson, Bull. Torr. Bot. Cl. 39:285. 1912.—Type: Bolivia, [La Paz, Franz Tamayo,] near Apolo, 6000 ft., *Williams 1303* (Holotype, US!; Isotypes, P!, US!).

Terrestrial fern with long-creeping, slender to rather stout, branched, solenostelic stem bearing stiff, dark brown, multicellular, basally pluriseriate bristles. Leaves remote, non-articulate, 0.3–1.5 m long. Petiole as long as to somewhat longer than lamina, with a single, gutter-shaped vascular bundle, adaxially convex to flattened-sulcate, with bristles at the base and sometimes further along the petiole. Lamina narrowly to broadly deltate, 1-pinnate-pinnatifid to bipinnate-pinnatifid, firm, catadromous, hypostomatic; pinnae (sub)sessile, subopposite, asymmetric, the basiscopic segments reduced, secondary pinnae and segments obliquely ascending, decurrent, ultimate segments deltoid-lanceolate, margin crenate to entire. Stomata paracryptic. Veins free, forked-subpinnate, catadromously branched. Sori terminal on a vein, protruding beyond the margin, directed downwards from plane of lamina, profusely paraphysate; sorus cyathiform to urceolate, laterally free; receptacle columnar. Sporangia on short, pluriseriate stalks; annulus almost vertical, continuous and 3/4 indurated. Spores trilete, 41–62 μm in diameter, slightly pitted to verrucate, mostly granulate.

Specimens Examined

Costa Rica. **Alajuela:** Shore of lake on Poás Volcano, 2500 m, *Lent 512* (F); Near edge of lake, Poás Volcano, 2500 m, *Lent 738* (F); Along shore of old crater lake of Volcán Poás, 2500 m, *Mickel 3654* (NY); Poás lake, 8500 ft., in sandy soil by lake shore, *Stork 2522* (US); Edge of small lake inside of an extinct sub-crater of Volcán Poás, about 11 km NW of Heredia, 2700 m, *Taylor 4496* (NY); Shore of lake, crater summit, Volcán Poás, 2600 m, *Weston 4624* (UC). **Heredia:** Volcán Barba, 6700 ft., *Evans 2660A* (MO, U, Z); Headwaters of Río Santo Domingo, ca. 3 km NE of San Rafael de Vara Blanca, N slope of Volcán Barba, 10° 11.5′ N, 84° 07′ W, 2060 m, *Grayum 7212* (MO, US); Bordes del Río Grande, afluente del Río Patria, en el Paso del Gallito, macizo del Volcán Barba, 2045 m, *Jimenez 2272* (F, NY, UC, US); Southeast shoulder, near end of Route 113, 6 miles NNE of San Rafael de Heredia, *Lloyd s.n.* (MO, UC); San Jose, 10 km N of San Rafael de Heredia on Volcán Barba, 1950 m, *Mickel 3001* (UC, US); Río de las Vueltas, 2100 m, *Goldgewicht 863* (US); En el Río de las Vueltas, 2000 m, *Goldgewicht 86369* (CR); End of rt. 113, where road crosses Río Patria about 100–200 m upstream, 2000 m, *Moran 3051* (AAU). **Limón:** N flank of Cerro Casma, along Ujarrás—San José Cabécar trail, Cordillera de Talamanca, 09° 20′ 30″ N, 83° 13′ 30″ W, 2250–2270 m, *Grayum 10329* (CR, F, MO); P.I. La Amistad Valle de Silencio, 09° 07′ 15″ N, 82° 57′ 55″ W, 2450 m, *Moraga 339* (CR, UC). **Prov. unknown:** *Wercklé 225* (P).

Colombia. **Nariño:** Municipio de San Francisco, carretera Pasto-Mocoa, entre El Mirador y San Francisco, 1500–2200 m. *Mora 4449* (COL). **Norte de Santander:** Municipio de Toledo, de Toledo

a Samore, 2200 m. *Lozano et al. 5472* (COL). **Putumayo:** road from San Francisco to Mocoa, km. 112 from Pasto, near El Mirador, 7200 ft. *Plowman 4352* (COL).

ECUADOR. **Morona-Santiago:** Limón-Gualacco Road, km 16, 2100–2200 m, montane forest, *Harling 24597* (QCA); Pica Sevilla de Oro-Mendez colecciones "entre Páramo del Castillo-Cerro Negro-Dominguillos", 78° 34′ W, 2° 47′ S, 3000–3250 m, *Jaramillo 5558* (AAU, QCA); Carretero Limón-Gualaceo, 35 km desde Olan de Milagro, Colecciones a lo largo de la carretera, 1800 m, *Navarrete 600* (AAU, QCA); Muletrack Sevilla de Oro-Mendez, eastern slope (E of Cerro Negro, El Castillo), montane forest along muletrack, 78° 34′ W, 2° 47′ S, 2800–2950 m, *Øllgaard 9479* (AAU, F); Sigsig, Chinguinda, 1500–2000 m, *Lehmann 5059* (K, US); Sapote, 2100 m, *Crespi 72* (US). **Azuay:** "Oriente" Border: Eastern Cordillera, between Oña and the Río Yacuambi, east slope, 8000–9000 ft., *Prieto 259* (F, GH, K, MO, NY, US). **Zamora-Chinchipe:** Road Loja-Zamora, km 14, 79° 09′ W, 4° S, 2750–2770 m, *Holm-Nielsen 3860* (AAU, F, MO, NY); Eastern slopes of the East Andes of Loja, 2000–2500 m, *Lehmann 5715* (K, US); Parque Nacional Podocarpus, around pass on road Loja-Zamora, 79° 07′ W, 3° 58′ S, 2750–2950 m, *Øllgaard 74387* (AAU, K, NY, QCA); Road Loja-Zamora, just E of the pass, low scrub along old trail, 79° 10′ W, 03° 58′ S, 2800 m, *Øllgaard 98820* (QCA); Strasse von Loja nach Zamora, Páramo del Nebula, Pass an der Grenze von Loja und Zamora-Chinchipe, 3600 m, *Schneider 579* (Z); 10 km outside Loja along new road Loja-Zamora, just past the Nudo, 2900 m, *van der Werff 9107* (AAU, MO, NY, UC); pass on road Loja-Zamora, ca. 15 km E of Loja, ca. 3005 m, *van der Werff 13456* (MO); Parque Nacional Podocarpus, Road Yangana-Valladolid, km 26, 79° 09′ W, 04° 29′ S, 2550 m, *Madsen 75754* (AAU, QCA); Parque Nacional Podocarpus, Road Yangana-Valladolid, just S and E of the pass (Nudo de Sabanilla), muletrack from pass toward Quebrada Honda, 78° 08′ W, 4° 27′ S, 2750–2950 m, *Øllgaard 91061* (GOET), *Øllgaard 90634B* (AAU, QCA). **Santiago-Zamora:** Trail between Pailas and El Pan, 2255–3445 m, *Steyermark 54324* (F, NY, US). (**Carchi:** Espejo, El Goaltal, Cerro Golondrinas, vegetacion arbustiva (matorral) espeso sobre la cresta de la montaña, bosque muy humedo montano bajo, 0° 51′N, 78 08′ W, 2600–2800 m, *Palacios and Clark 12463* (MO); not seen by us, but studied by A.R. Smith, pers. com.).

PERU. **Huanuco:** Southwestern slope of the Río Llulla Pichis watershed, on the ascent of Cerras del Sira, 9° 25′ S, 74° 42′ W, 2205 m, *Dudley 13420* (GH); Playapampa, ca. 9000 ft., *Macbride 4521* (F, GH, US). **Pasco:** Border Prov. Oxapampa and Pasco, 2700 m, *van der Werff 8564* (MO, NY). **Cuzco:** Cachayocpata, entrada del Valle San Miguel La Convención, 10000 ft., *Bües 2119* (US); La Convención 73° 34′ W, 12° 37′ S, 2555 m, *Dudley 10713* (GH, MO).

BOLIVIA. **La Paz:** Nor Yungas, 5 km de Chuspipata hacia Coroico, 16° 23′ S, 67° 48′ W, 2750 m, *Kessler 11981* (UC, GOET); Nor Yungas, Chuspipata, camino sobre loma de montaña a Coroico, 16°18′ S, 67°49′ W, 3000 m, *Lehnert 032* (LPB, UC, GOET); Nor Yungas, entre Cotapata y Chuspipata, 16° 18′ S, 67° 50′ W, 3100 m, *Smith 13139* (F, MO, UC). **Cochabamba:** José Carrasco Torrico, 112 km antigua carretera Cochabamba-Villa Tunari, 17° 07′ S, 65° 38′ W, 2700 m, *Kessler 6928* (UC); José Carrasco Torrico, 130 km antigua carretera Cochabamba-Villa Tunari, 17° 07′ S, 65° 36′ W, 2000 m, *Kessler 7209* (UC, GOET).

ACKNOWLEDGEMENTS

We thank Erika Hofmann, Ulrike Geisler and Tanja Siemsen for collaboration on specimen analysis, Gabriele Weiss for the SEM photographs, A. R. Smith for comments on the manuscript, and the following herbaria and their curators for making material available to us: AAU, COL, CR, F, GH, GOET, K, MO, NY, P, QCA, U, UC, US, Z (acronyms according to Holmgren *et al.*, 1990).

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Growth, Leaf Characteristics, and Spore Production in Native and Invasive Tree Ferns in Hawaii

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ABSTRACT.—The Australian tree fern *Sphaeropteris cooperi* is an invasive species in Hawaiian wet forests where it displaces *Cibotium*, the dominant native Hawaiian tree fern, where they co-occur. This study was undertaken in order to assess the relative growth rates and reproductive potential of *S. cooperi* and the native *Cibotium* species. Field measurements of growth rates, fertile frond production and leaf traits were made monthly over the course of one year. *Sphaeropteris cooperi* had a significantly higher growth rate, both in terms of height increase and frond production, and maintained four times more fronds than the native *Cibotium* species. The mean annual height increase of the invasive tree fern was 15 cm compared to 2 to 3 cm for the native tree ferns. The leaf mass per area of *S. cooperi* was significantly lower than that of the native *Cibotium* species, and the leaf life span was significantly shorter, suggesting that the cost of construction of the invasive species' fronds was relatively low. *Sphaeropteris cooperi* also produced significantly more fertile fronds per month than the native tree ferns. These differences in life history characteristics may help explain the rapid spread and success of *S. cooperi* in Hawaii.

The invasion of non-native plants into native ecosystems has become a topic of great concern in recent years, particularly in isolated island ecosystems such as the Hawaiian Islands (Loope and Mueller-Dombois 1989, Vitousek et al. 1987). Of the more than 800 introduced plant species which have become naturalized in the Hawaiian Islands, 30 are ferns (Vitousek et al. 1987, Wagner 1995). The Australian tree fern, *Sphaeropteris cooperi* (Hook. ex F. Muell.) Tryon [syn. Cyathea cooperi (Hook. ex F. Muel.) Dom.], was introduced to the Hawaiian Islands as a horticultural plant and first escaped from cultivation in the 1950s (Wagner 1995). *Sphaeropteris cooperi* is now naturalized on the islands of Oahu, Maui, Kauai, and Hawaii (Wagner 1995) and is listed as among the worst alien plant invaders of Haleakala National Park (Loope et al. 1992).

Tree ferns in the genus *Cibotium* are the dominant native tree ferns in Hawaii. There are four endemic species in this genus, three of which (*C. chamissoi* Kaulf., *C. menziesii* Hook., *C. glaucum* [Sm] Hook. and Arn.) are found on all of the major islands. The fourth (*C. nealii* Degener) is found only on the island of Kauai (Palmer 1994). These endemic ferns grow in semi-wet to wet forests from an elevation of 40 m to 1800 m, and at a mean annual temperature range between 13°C and 23°C (Becker 1976). In the Hawaiian forests, tree ferns are keystone species which have a substantial impact on the structure and function of the forest around them. For example, the native *Cibotium* support native Hawaiian plants as epiphytes (Medeiros et al. 1993), as well as serving as nurse logs for a number of native species which become established on the well-aerated adventitious roots of the tree ferns' trunk (Buck 1982). The native

TABLE 1. Elevation (m), annual precipitation (mm), tree fern species present, and the number of individuals studied (n) at the sites used for this study. Lyon Arboretum, Wa'ahila Ridge and Mt. Ka'ala are on the island of O'ahu, Waineke Swamp is on the island of Kauai.

Site	Elevation (m)	Rainfall (mm)	Species	Sample size (n)
Low elevation (Lyon Arboretum)	155	4000	*Sphaeropteris cooperi*	6
			Cibotium chamissoi	8
Mid elevation (Wa'ahila Ridge)	430	2000	*Cibotium chamissoi*	6
			Cibotium menziesii	6
High elevation (Mt. Ka'ala bog)	1225	2000	*Cibotium chamissoi*	8
			Cibotium menziesii	8
			Cibotium glaucum	8
High elevation (Waineke Swamp)	1250	2000	*Sphaeropteris cooperi*	6

tree ferns also sequester high levels of nitrogen and phosphorus in their leaves compared to other native Hawaiian rainforest species (Vitousek et al. 1995).

This study was undertaken in order to assess the relative growth rates and reproductive potential of the native *Cibotium* and the invasive *Sphaeropteris cooperi*. We hypothesize that the invasive *S. cooperi* has faster growth rates and a greater spore production than the native tree ferns. In addition, we hypothesize that the invasive tree ferns produce fronds and spores more consistently throughout the year than the native ferns. This difference in growth and reproductive capacity may contribute to the ability of *S. cooperi* to outcompete the native tree ferns in Hawaiian rainforests. We also predict that the invasive tree fern, consistent with what has been observed in other invasive species in Hawaii (Baruch and Goldstein 1999), will have a shorter leaf life span and lower leaf mass per area (LMA) than the native tree ferns. The native *Cibotium* species were chosen for comparison based on their similar growth form and phylogenetic relatedness to the Australian tree fern. By studying native and invasive species of similar growth form that grow in the same habitat, predictions as to the relative growth and reproductive performance of the invasive and native plants can be drawn.

MATERIALS AND METHODS

STUDY SITES.—Four sites were selected for this study based on accessibility and species composition (Table 1). The range of elevations of the sites, from 200 m to 1250 m, encompasses much of the elevational range of the native Hawaiian tree ferns. The tree ferns at each site were growing under similar substrate and environmental conditions. All sites were tree dominated with an understory of ferns and tree ferns. The tree ferns at the two high elevation sites were located in relatively well drained soils at the edge of a bog. Individual plants of the same size class (1.5 m to 2.0 m) were randomly selected, and comparisons were made between individuals of the invasive and native tree ferns from the shaded understory. Six to eight individuals of each species at each

site were studied (Table 1). The low elevation site was the only accessible site with sympatric native and invasive tree ferns, and the only site where the invasive tree fern was available for a long enough period of time to measure growth. The high elevation site on Oahu contained only native tree ferns. An additional site on Kauai with similar elevation and rainfall was selected for comparison. *Sphaeropteris cooperi* is naturalized at this site, but *Cibotium chamissoi, C. menziesii,* and *C. glaucum* were not found. Due to management efforts to control *Sphaeropteris cooperi,* this plant was not available at all sites. This fact, and the fact that sample sizes were relatively small, led us to be cautious when making extrapolations based on the results of this study.

GROWTH MEASUREMENTS.—Stem growth rate, frond production, and leaf life span were monitored for 12 consecutive months, from November 1997 through October 1998, on mature sporophytes of the same size class at the Lyon Arboretum, Waahila Ridge and Mt. Kaala sites. It was not possible to measure growth and leaf life span on the high elevation population of *S. cooperi* as management efforts to control this plant resulted in their removal before measurements could be completed. A point at the soil surface on one side of each tree fern was marked and measurements of stem height from the meristem to this point were taken monthly. All newly emerging fronds were flagged, dated and monitored until senescence. The number of months between frond emergence and senescence was recorded as the life span of the frond.

LEAF TRAITS.—The total number of living fronds was counted monthly. For the purposes of this study, a frond was defined as living when it had more than 10% green tissue remaining. The mean surface area per frond was estimated by multiplying the total number of pinnules per frond by the average surface area of a pinnule. Ten pinnules per frond were collected, and the surface area of each pinnule was measured with a Licor 3000A area meter (Li-Cor, Inc., Lincoln, Nebraska) and averaged. The surface area of the tip of each frond was measured separately, as the pinnules in this region were smaller. The average surface area per frond was multiplied by the number of mature living fronds per plant to estimate the total leaf surface area per plant.

Tissue samples from the middle of a frond were collected in the field for leaf mass per area (LMA) estimates. Samples were kept in a cooler for less than 24 hours until they could be returned to the laboratory, at which time the area was measured using a LI-3000A area meter. The leaf samples were then dried at 60°C for five days and weighed to determine LMA (the ratio of leaf dry weight to leaf surface area).

The number of fronds with spores was counted each month from November 1997 through October 1998. Each frond was examined to insure that sori were intact, and no fronds with dehisced sporangia were counted as fertile.

STATISTICAL ANALYSIS.—A one-way ANOVA among all the samples was performed, followed by pre-planned contrasts (Moore and McCabe 1993). Contrasts were used to assess for significant differences in LMA and leaf life span between native and invasive species at low elevation and at high elevation,

and between all natives and the invasive regardless of elevation. Monthly frond production was standardized (number of new fronds produced in a month divided by the maximum number of fronds on the plant). The standardized monthly frond production was used to calculate a standard error of frond production for each species at each site. This standard error was used as a measure of seasonal variation in leaf production (Stratton et al. 2000). The same was done for spore production. Repeated measures ANOVA's were used to analyze yearly data on new frond production, leaf surface area, height growth increase, and spore production. A Tukey's pairwise test was used to determine significance between species.

Results

The mean height growth of the invasive tree fern *S. cooperi* over the course of one year was significantly greater ($P<0.001$) than the mean height increase of native tree ferns (Fig. 1). After 11 months of measurements, the mean growth of the invasive tree fern was 15.4 cm, while that of the native tree ferns ranged from 1.9 cm to 3.0 cm. The most valid comparison is between *S. cooperi* and *C. chamissoi* at the same site, where the native tree fern had a mean annual growth of 3.0 cm compared to the 15.4 cm of the invasive tree fern ($P<0.001$). There were no substantial site-specific differences in height growth among the native tree fern species. An increase in growth during the months of April to August corresponded to an increase in rainfall during this period (Fig. 1).

The number of new fronds produced per month by *S. cooperi* was significantly greater ($P<0.001$) than the number of new fronds produced by the native tree ferns (Fig. 2). *Sphaeropteris cooperi* produced an average of 2.7 new fronds each month, while the native tree ferns at the same elevation produced an average of 0.4 new fronds per month, and all native tree ferns regardless of elevation produced an average of 0.2 to 0.4 new fronds per month. The native tree ferns also exhibited greater seasonal variation in frond production than *S. cooperi* (Table 2). Seasonality, calculated as the standard error of the standardized monthly frond production, was 2.5 to 3.5 times greater in the native tree ferns compared to *S. cooperi.* A peak in new frond production was observed for the native tree ferns in April, and again in August/September (Fig. 2), however no corresponding peak in leaf surface area per plant was observed as leaf senescence often corresponded with leaf expansion. *Sphaeropteris cooperi* maintained from 12 to 18 fronds per plant over the course of one year. The native tree ferns at all elevations maintained only 3 to 6 fronds per plant over the same time period (data not shown). As a consequence, *S. cooperi* had a higher leaf surface area per plant than the native tree ferns (Fig. 2).

The fronds of low elevation *S. cooperi* had a significantly lower LMA than the native tree ferns growing at the same elevation ($P<0.001$). Similarly, high elevation *S. cooperi* had a significantly lower LMA than that of native tree ferns growing at high elevation ($P<0.001$). The fronds of *S. cooperi* had a 1.3 to 3.8 times lower LMA than that of the natives at all sites (Table 2). In addition, the LMA of the native tree ferns tended to increase with increasing ele-

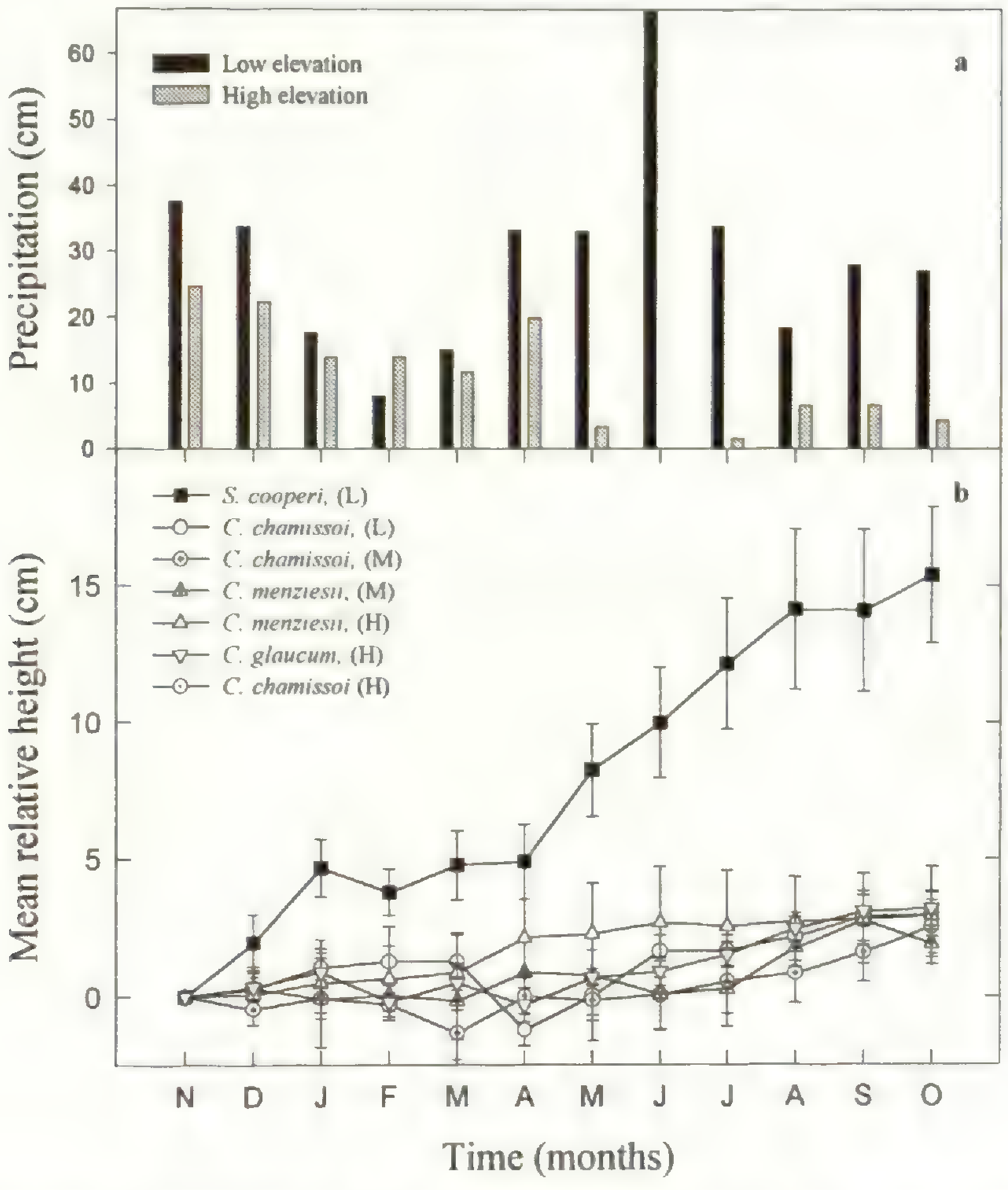

FIG. 1. Seasonal variation in rainfall at the low elevation site (Lyon Arboretum) and high elevation site (Mt. Kaala) on the island of Oahu (a), and mean relative height growth of native and invasive tree ferns (b) over the course of one year, from November 1997 through October 1998. Tree fern height in November was used as a reference. Symbols represent mean ± SE (n = 6 to 8). Negative values represent a decrease in height as croziers emerge from the trunk. Filled symbols represent the invasive tree fern *Sphaeropteris cooperi*, and open symbols represent the three native species in the genus *Cibotium*. L, M, and H indicate low, middle, and high elevation study sites. Precipitation data from the National Weather Service.

vation. The leaf life span of low elevation *S. cooperi* was significantly shorter than the leaf life span of the low elevation native tree ferns ($P<0.001$). The invasive tree fern had 45 to 50 percent shorter leaf life spans than the native tree ferns at all elevations (Table 2).

The number of fertile fronds per plant was significantly greater ($P<0.001$) in *S. cooperi* than in the native tree ferns (Fig. 3). The invasive tree fern had from 9 to 15 fertile fronds per month, which represented 60 to 75 percent of the total number of fronds per plant. The native tree ferns had between 1 and 4 fertile fronds per month, which represented 20 to 60 percent of the total number of fronds per plant. In addition, the native tree ferns exhibited greater seasonal variation in fertile frond production than *S. cooperi* (Table 2). Seasonality, calculated as the standard error of the standardized mean monthly

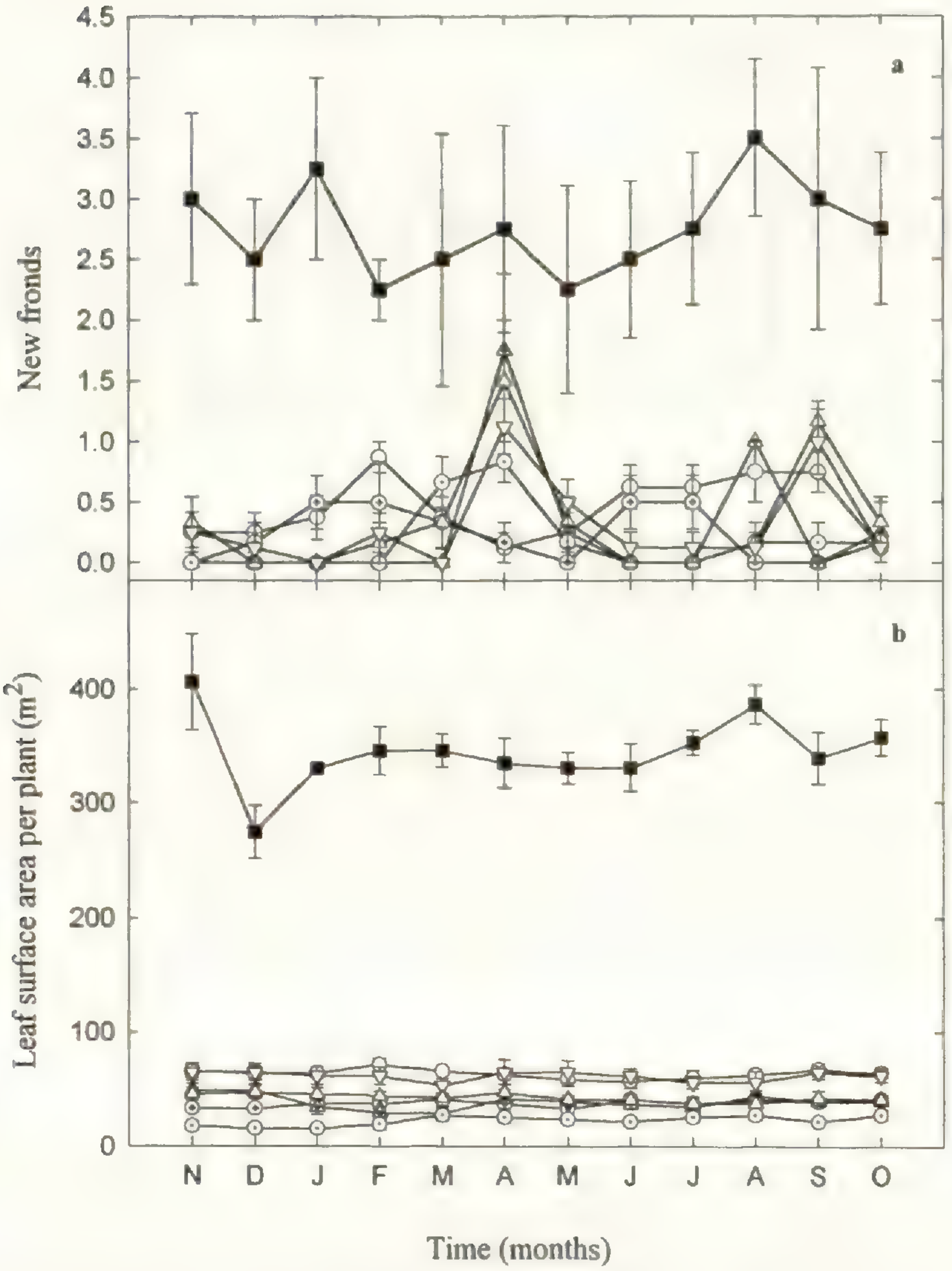

FIG. 2. Number of new fronds produced each month (a) and total surface area per plant (b), from November 1997 through October 1998. Symbols are mean ± SE (n = 6 to 8). Symbols as in Fig. 1. Absence of error bar indicates that the SE is smaller than the symbol.

number of fertile fronds produced per month, was between 20 and 50 percent higher in the native tree ferns (Table 2).

DISCUSSION

Native tree ferns on Oahu (this study) and the island of Hawaii (Walker and Aplet 1994, Wick and Hashimoto 1971) had relatively low annual height increases compared to the invasive tree fern. The Australian tree fern grew very little in January through March, when monthly precipitation was below 20 cm. Growth increased in April through August, apparently as a result of higher precipitation. Growth in the native tree ferns at the low elevation site also increased in April. At the high elevation site we noted that growth showed

TABLE 2. Leaf mass per area (LMA), leaf life span, standard error (SE) of standardized leaf production and of standardized spore production for native and invasive tree ferns at three elevations. Standard error of leaf production is a measure of seasonal variability in leaf production. Standard error of standardized spore production is a measure of seasonal variability in spore production. Values are means ± SE. Symbol (†) next to a grouping indicates statistical significance ($P \leq 0.01$) between the invasive and native ferns within that group.

		LMA ($g\ m^{-2}$)	Leaf life span (months)	SE of leaf production	SE of spore production
Low elevation					
Invader	*S. cooperi*	37.9 ± 2.1†	6.0 ± 0.15†	3.35	1.97
Native	*C. chamissoi*	54.7 ± 2.8	11.0 ± 0.30	8.49	3.21
Mid elevation					
Natives	*C. chamissoi*	50.9 ± 3.1	12.0 ± 0.71	11.7	3.66
	C. menziesii	78.9 ± 4.0	10.8 ± 1.1	8.32	2.45
High elevation					
Invader	*S. cooperi*	34.7 ± 1.8†	n/a	n/a	n/a
Natives	*C. chamissoi*	120.3 ± 7.2	10.6 ± 0.42	9.02	3.46
	C. menziesii	127.5 ± 3.8	12.0 ± 0.50	8.72	2.63
	C. glaucum	135.2 ± 3.0	12.6 ± 0.73	8.94	4.04

less seasonal variation and may have been influenced by higher irradiance during drier months, when cloud cover is relatively low, as well as by rainfall patterns. Frond production of the native tree ferns also exhibited seasonal variation, with the majority of frond production occurring from February through April. There was virtually no frond production during the months of October, November, and December. This seasonal pattern was similar to the pattern of frond production observed in *Cibotium* on the island of Hawaii by Wick and Hashimoto (1971). In neither study did the pattern of frond production appear to correspond with precipitation. In contrast to the native tree ferns, frond production in the Australian tree fern was consistent throughout the year. The faster growth rate and greater and more consistent frond production of the Australian tree fern *S. cooperi* could contribute to its ability to outcompete the native Hawaiian tree ferns.

When plants of the same growth habit are compared, plants with a higher leaf surface area tend to have a higher relative growth rate (Lambers et al. 1998). *Sphaeropteris cooperi* had over three times more fronds per plant than the native tree ferns, and over four times more leaf surface area (Fig. 2). A greater leaf surface area per plant, along with a greater annual height increase, could allow the Australian tree fern to intercept more light than the native tree ferns, and thus potentially fix more carbon. In a previous study, we observed that the photosynthetic rate at light saturation for shaded plants was between 5 and 7 $\mu mol\ m^{-2}\ s^{-1}$ for the invasive tree fern, while the photosynthetic rate at similar light levels was between 3.4 and 4 $\mu mol\ m^{-2}\ s^{-1}$ for the native tree ferns (Durand and Goldstein 2001). The pattern of allocation of carbon to different plant organs can also affect the annual height increase of the plant. The native tree ferns have a high starch content in their trunk, and were once harvested for this starch (Ripperton 1924, Wick and Hashimoto

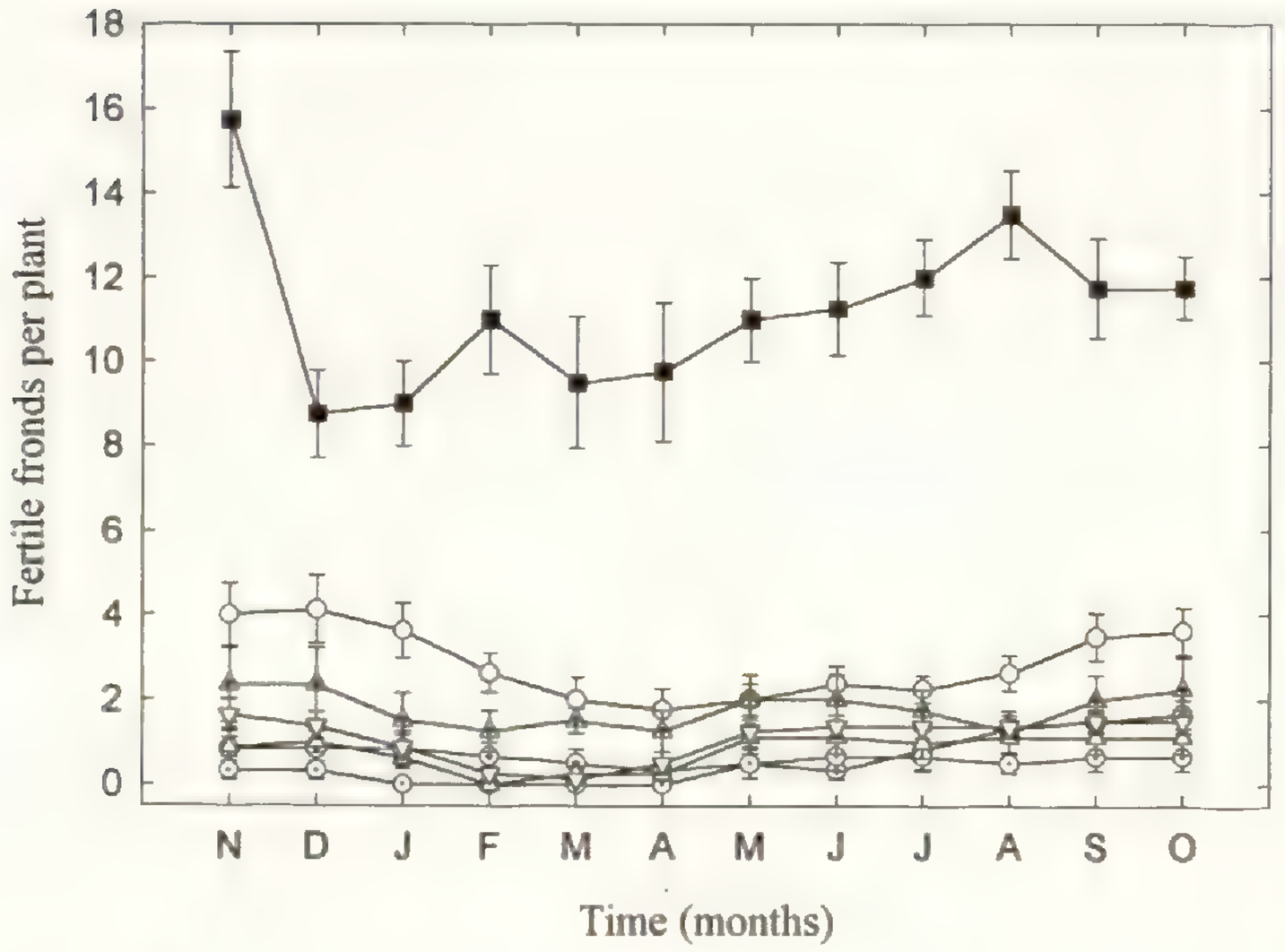

FIG. 3. The number of fertile fronds (with spores) per plant of native and invasive tree ferns, measured from November 1997 through October 1998. Symbols represent ± SE (n = 6 to 8). Symbols as in Fig. 1.

1971). While no data is available on the trunk starch content of *S. cooperi*, in areas where *S. cooperi* and *C. chamissoi* grow together, wild pigs preferentially feed on the trunk starch of the native tree ferns. Thus, another possible explanation for the low annual height increase of the native tree ferns is a relatively large allocation of carbon, in the form of starch, to the fern's trunk.

The native tree ferns exhibited seasonal changes in fertility, with the greatest number of fertile fronds present in the months of September through February. The invasive tree fern did not exhibit as much seasonal variation in fertility, though there were more fertile fronds per plant in the months of July through November. The difference in seasonal spore production may allow spores of the Australian tree fern to germinate at a time when spores of the native tree ferns are not available. It has been suggested that successful invasive species either utilize resources more efficiently, or at a time when natives are inactive or unable to access the resources (Vitousek 1986). Consistent monthly spore production could give the Australian tree fern a reproductive advantage over the native tree ferns.

Ferns in the genus *Sphaeropteris* produce 64 spores per sporangium (Gastony 1974), as do ferns in the genus *Cibotium* (Sporne 1966). Dyer (1979) estimated that an average fertile frond of *Cibotium chamissoi* produces 700,000,000 spores. With between 1 and 4 fertile fronds produced per plant per year (Fig. 3), spore production per year ranges between 700 million and 2.8 billion spores. *Sphaeropteris cooperi* produced between 22 and 27 fertile fronds per plant per year (Fig. 3), though the number of sporangia produced per frond is not known. However, this species has a greater frond surface area than *Cibotium*, and given the equal number of spores per sporangia in *Cibotium* and *Sphaeropteris*, assuming equal spore production per frond seems a

conservative estimate. If *S. cooperi* does indeed produce an equal number of spores per frond as *Cibotium chamissoi,* this fern has the potential to produce 15.4 billion to 18.9 billion spores per year. While both species produce a large number of spores each year, *S. cooperi* can potentially produce 13 to 16 billion more spores per year. Studies of the spore production of the invasive tree fern, and spore viability of both the native and invasive tree ferns, need to be conducted in order to have a more complete understanding of the relative reproductive capacity of these ferns.

Variation in leaf life span has been found to be an important predictor of numerous plant responses (Chabot and Hicks 1982, Reich *et al.* 1992, Reich *et al.* 1997). The leaf life span of higher plants can range from less than one month to 25 years (Chabot and Hicks 1982). When compared across diverse ecosystems and biomes, a short leaf life span is associated with factors such as higher photosynthetic rate, higher leaf nitrogen content, lower leaf construction cost, and a lower LMA (Chabot and Hicks 1982, Reich et al. 1992, Reich 1993, Reich et al. 1997). The mean leaf life span of the Australian tree fern was significantly shorter than the mean leaf life span of the native tree ferns (Table 2). As expected for a plant with a shorter leaf life span, the Australian tree fern had a significantly lower LMA than the native tree ferns (Table 2). Generally, longer-lived leaves require more secondary compounds to protect the leaves against herbivores and pathogens, whereas shorter-lived leaves with a low LMA are able to maximize photosynthetic return per unit weight of the leaves (Lambers et al. 1998, Reich 1993). The Australian tree fern produced short-lived leaves with high leaf nitrogen and high photosynthetic capacity (Durand and Goldstein 2001) at a relatively low carbon cost to the plant compared to the native tree ferns. In a recent study of 34 native and 30 invasive higher plants in the Hawaiian Islands, it was found that the latter had lower LMA, higher leaf nitrogen, and higher photosynthetic rates (Baruch and Goldstein 1999). The patterns observed with the ferns in this study were consistent with the pattern found in higher plants, suggesting that this suite of traits are important determinants of the success of invasive species in the Hawaiian Islands, for both ferns and higher plants alike.

The difference in leaf life span, LMA, growth rate, and spore production between the invasive and native tree ferns suggests that there are differences in life history characteristics. Tree ferns in Costa Rica that grew as pioneer species in secondary forest had higher leaf turn-over rates, faster growth rates, and more rapid production of sori than tree ferns that grew in primary forest (Bittner and Breckle 1995). Similarly, the high leaf turn-over rate, high spore production, and rapid growth rate in *S. cooperi* suggests that it tends to behave as a pioneer, or r selected species. The native tree ferns, on the other hand, had a much lower leaf turnover rate and slower growth rates, as well as lower reproductive output. This difference in life history characteristics may partially explain why *S. cooperi* can become established and spread quickly in Hawaiian rainforests, particularly in areas of disturbance.

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Shorter Note(s)

Binomial for *Dryopteris clintoniana* × *goldiana*.—One strikingly handsome *Dryopteris* hybrid, the largest of all North American temperate *Dryopteris* (Thorne, F. and L. Thorne. 1989. *Henry Potter's Field Guide to the Hybrid Ferns of the Northeast.* Vermont Institute of Natural Science, Woodstock, VT) and an excellent fern for hardy gardening (Mickel, J. T. 1994. *Ferns for American Gardens.* Macmillan, New York, NY), is without a collective epithet: *D. clintoniana* × *goldiana.*

Dryopteris ×mickelii Peck, *hyb. nov.*—TYPE: UNITED STATES, New Jersey, Sussex Co., west side of extensive swamp south of Big Spring, 3 km south of Springdale, 17 Oct. 1969, *James D. Montgomery 90895n* (NY).

Planta hybrida inter Dryopterem clintonianam et D. goldianam, aliis characteribus inter parentes media, sporis abortivis.

The hybrid is named to honor Dr. John Thomas Mickel (1934–) for his many endeavors on North American fern taxonomy, floristics, and horticulture, particularly his promotion of gardening with hardy ferns. The hybrid is distinguished readily from its parents by its abortive and irregular sized spores, intermediate frond morphology, and shared characters with both parents (see table in Thorne and Thorne, 1989, page 34). *Dryopteis ×mickelii* has fronds up to 160 cm long, stipe to frond length ratio of 1:4, and is covered moderately with brown to dark brown scales. The blade is about 120 cm long, with a width to length ratio of about 2:5, with a very long and broad outline. The pinnae are regularly spaced, ascending relative to the rachis, with pinna shape wide, long rectangular and acuminate. Sori are borne close to the pinnule midrib. *Dryopteris ×mickelii* shares with *D. clintoniana* the following features: dark coloration at base of scales, relative length of blade, pinnae ascending from rachis, relatively narrow blade, and intermediate sorus location. *D. ×mickelii* shares with *D. goldiana* the following features: wide blade, length of pinnules, falcate pinnules, relatively dark scales, and shape of pinnae.

This hybrid occurs in rare and local populations in southern Ontario, south to New York, New Jersey, and Pennsylvania, and westward as outliers in Michigan and Ohio. This relatively narrow geographic range reflects the geographic overlap of the two parental ranges in the northeastern region of North America. The habitat of wetland woods and swamps were more common in early postglacial times, but have declined since then, particularly in the western one-half of the range. In nature, the hybrid warrants conservation efforts wherever it still occurs.

PARATYPES.—CANADA. **Ontario**: in woods at Ottawa, *Scott s. n.* (NY). UNITED STATES: **Michigan**: Washtenaw Co.: deep yellow birch swamp in Irwin's woods, *Wagner 9458* (US); Tuscola Co.: south of Murphy Lake, *Wagner 63051* (US); tamarack swamps at Oxford, *Farnwell 6117* (US). **New Jersey**:

Sussex Co.: swamp west of Springdale at Big Spring near Newton, *Dowell 4929* (NY, US), *Dowell 5033* (NY); Sussex Co.: roadside 1 mi south of Greendell, *Edwards s. n.* (NY); Bearfort Mtn., *W. D. Miller 1648* (NY); West Englewood, *Carhart 2b* (NY) 2 sheets. **New York:** Green Lake, Jamesville, *W. R. Dunlop s. n.* (NY); Harris Swamp near Pilot Knob, *Benedict s. n.* (US-2202218); Kirkville, *L. M. Underwood s. n.* (NY); Staten Island: Arlington Station, *Dowell 2801a & b* (US). **Ohio:** Geauga Co.: *Hopkins s. n.* (NY). **Pennsylvania:** Berks Co.: swamp along spring-run 1.5 mi ne of Bernharts, *Wherry s. n.* (US-1849217); Delaware Co.: valley of Cruise Creek, *Poyser 1286* (NY); Wakefield, Lanbor, *J. J. Carter s. n.* (NY). **Vermont:** N. Lynnfret (Limfret?), *A. P. & L. V. Morgan s. n.* (US-154517).

In the garden, its vegetative propagation should be promoted and supplemented with modern tissue culture techniques to meet horticultural demands. It is a large and vigorous fern that forms extensive colonies through vegetative expansion while under cultivation. One specimen from New York was planted at the New York Botanical Garden, Bronx, NY in 1960 by members of the American Fern Society. Thirty-five years later, that plant had formed a clone 4 m across with over 200 apices (Mickel, 1994). JAMES H. PECK, Department of Biology, University of Arkansas at Little Rock, 2801 S. University Ave., Little Rock, AR 72204.

Cryopreservation of Shoot Tips of *Selaginella uncinata*.—Cryopreservation, or storage in liquid nitrogen (LN), has been successful for a wide variety of plant tissues, including shoot tips of *in vitro* cultures of higher plants (Bajaj, *In* Y. P. S. Bajaj, *Cryopreservation of Plant Germplasm I, Biotechnology in Agriculture and Forestry* 32. Springer, Berlin, 1995). At the temperature of LN, −196°C, long-term, stable storage of rare, endangered, or other valuable plant germplasm can be achieved. In an attempt to extend this technology to pteridophytes, LN storage of shoot tips of *in vitro* grown *Selaginella uncinata* was tested using the encapsulation dehydration procedure (Fabre and Dereuddre, Cryo-Letters 11:413–426, 1990).

Shoot cultures of *Selaginella uncinata* (Desv. ex Poir.) Spring. were established from a plant purchased from Carolina Biological Supply Co. (voucher specimens deposited at the University of Cincinnati Herbarium, CINN, and at the CREW Herbarium). Tissues were surface disinfested in a 1:20 dilution of commercial sodium hypochlorite for five minutes, followed by two rinses in sterile, ultrapure water. The tissues were then transferred to a basal medium consisting of half-strength Murashige and Skoog salts with minimal organics (MS medium; Linsmaier and Skoog, Physiol. Plant. 18:100–127, 1965) with 1.5% sucrose and 0.22% Phytagel (Sigma Chemical Co.). Cultures were maintained on this medium in 60 × 15 mm plastic petri plates, approximately 15 ml of medium/plate, at 26°C under Cool White fluorescent lights with a 16:8 hr, light:dark cycle. For preculture, one week prior to freezing the tissues were transferred to fresh basal medium or to basal medium plus 10 μM abscisic acid (ABA), which was filter sterilized and added to the medium after autoclaving.

TABLE 1. Survival of shoot tips of *S. uncinata* through the steps of the encapsulation dehydration technique, 3–15 clean shoot tips per treatment. Encapsulated shoot tips were pretreated in 0.75 M sucrose, dried and exposed to LN, and samples were cultured after each step. Results are the mean of three experiments (±SE), except for long term LN exposure, for which there was one trial. Numbers of samples per trial are given in parentheses.

	Percent survival[1]			
Preculture medium	Pretreated	Dried	1 hr LN exposure	3.5 yr LN exposure
Basal	79 ± 7[a] (12, 13, 13)	25 ± 6[bc] (12, 13, 10)	11 ± 7[b] (8, 15, 11)	0 (14)
Basal + ABA	90 ± 6[a] (12, 9, 5)	92 ± 8[a] (12, 7, 5)	57 ± 18[ac] (3, 9, 6)	44 (11)

[1] Values with different superscripts are significantly different to the 0.05 level.

For cryopreservation, the procedure of Fabre and Dereuddre (1990) was followed. Shoot tips approximately 2–3 mm in length were excised and placed in a solution of 3% alginic acid with 0.75 M sucrose in calcium-free MS medium. The solution containing the tips was then added dropwise to a solution of 100 mM $CaCl_2$ to form the alginate beads. These were incubated for 18 hr in liquid MS medium containing 0.75 M sucrose and were then dried for 4 hours in open dishes on filter paper under the air flow of the laminar flow hood. The dried beads containing the shoot tips were then transferred to 2 ml polypropylene cryovials and immersed directly into LN. After 1 hr, the cryovials were removed from the LN and allowed to warm at room temperature for 20 minutes. The encapsulated shoot tips were then transferred to basal medium for rehydration and recovery growth. Survival was measured as regrowth from a shoot tip. Results were analyzed by ANOVA (Tukey HSD Test, StatSoft, QuickSTATISTICA software).

Drying and LN exposure significantly decreased survival from shoot tips which were precultured on basal medium alone (Table 1). When shoots were precultured for one week on basal medium plus 10 μM ABA, there was no decrease in survival when the tissues were dried, although survival did decrease to approximately half when the tissues were exposed to LN. However, survival of both dried and LN exposed tissues was significantly better than that of control tissues.

Shoot tips which were prepared using the encapsulation dehydration procedure with and without ABA preculture were also transferred to LN for long-term storage. When these were removed after 3 1/2 yrs of storage in LN and recultured on basal medium, there was no regrowth from shoot tips which had been isolated from tissues grown on basal medium. However, 44% of the shoot tips which had been precultured on basal medium plus ABA initiated good growth after eight weeks (Figure 1).

These results demonstrate that shoot tips of *S. uncinata* can survive LN exposure and long-term storage in LN when they are prepared using the encapsulation dehydration procedure in combination with preculture on ABA.

FIGURE 1. Shoot tip of *S. uncinata* growing out of alginate bead, 8 weeks after exposure to LN. 6.7 X

Encapsulation dehydration is a technique which has been successful with shoot tips of a number of species of angiosperms (Touchell and Dixon, *In* B. G. Bowes. *Atlas of Plant Propagation and Conservation.*, Manson Publishers, London, 1999) as well as with gametophytes of ferns and bryophytes (Pence, The Bryologist 101:278–281, 1998 and Amer. Fern J. 90:16–23, 2000), and it is not surprising that it can also be successful with shoot tips of the fern allies as well.

Survival of *S. uncinata* through cryopreservation was low unless the tissue was precultured on ABA. Treatment with ABA has been shown to initiate desiccation tolerance in the fern *Polypodium virginianum* L. (Reynolds and Bewley, J. Exper. Bot. 44:921–928. 1993a and J. Exper. Bot. 44:1771–1779, 1993b), as well as in bryophytes (Hellwege et al., Planta 198:423–432, 1996; Pence, 1998), and ABA is involved in several aspects of desiccation tolerance and water stress in seed plants (Hartung and Davies, *In* W. J. Davies and H. G. Jones, Abscisic Acid. Physiology and Biochemistry. BIOS Scientific Publishers, Oxford, 1991). It appears that preculture on ABA improves tolerance of *S. uncinata* shoot tips to the stresses of drying and LN exposure in the encapsulation dehydration procedure, perhaps mimicking a natural response to stress in this species. Several other species of *Selaginella* are known to be "resurrection plants," with a remarkable ability to tolerate extreme water loss

(see Proctor and Pence, *In* M. Black and H. Pritchard. *Desiccation and Plant Survival*. CAB International, Oxon, U.K., in press).

Although encapsulation dehydration was successful in preserving shoot tips of *S. uncinata,* preliminary studies in this laboratory indicate that the survival of some other species of *Selaginella* through this procedure may not be as high (unpublished results). Species differ in their ability to survive LN exposure, and other techniques, such as slow freezing or vitrification, may be more successful with other species in this genus (Fukai et al., Euphytica 56:149–153, 1991; Yamada et al., Plant Science 78:81–87, 1991).

There are over 700 species of pteridophytes which are of conservation concern worldwide, including 23 species of *Selaginella* (Walter and Gillett, 1997 IUCN Red List of Threatened Plants. IUCN—The World Conservation Union, Gland, Switzerland 862 Pp., 1998). Further studies should reveal how broadly applicable the encapsulation dehydration procedure is to shoot tips of pteridophytes and how cryopreservation may be used for preserving *Selaginella* germplasm for research and conservation.—VALERIE C. PENCE, Center for Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220.

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Systematics of the Northern Andean *Isoëtes karstenii* Complex

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ABSTRACT.—The *Isoëtes karstenii* complex includes those species characterized by laevigate megaspores, acute to free ala apices, a highly reduced labium, and distributions in the high altitude páramos of Venezuela, Colombia, and Ecuador. These species have been variously treated in the literature. Many early authors placed all Andean laevigate-spored specimens in *Isoëtes lechleri*, a central Andean species, while one later author recognized up to eight northern Andean species distinct from *I. lechleri*. This research represents a revisionary study to determine the number and identity of the taxa in this complex. Multivariate and descriptive analyses of morphological data and chromosome counts were performed. Five species are recognized including the previously described *I. karstenii* and *I. palmeri*. One variety is transferred from *I. lechleri* to *I. karstenii* (*I. karstenii* var. *anomala*). Three of the recognized species are newly described: *I. fuliginosa*, *I. hemivelata* and *I. precocia*. Chromosome counts revealed that *I. karstenii* and *I. precocia* are diploids ($2n = 22$) and that *I. palmeri* is tetraploid ($2n = 44$). Estimates of chromosome number based on spore size for *I. fuliginosa* and *I. hemivelata* indicate that they are polyploid ($2n \geq 44$).

The laevigate-spored taxa of *Isoëtes* L. from the northern Andes have long presented taxonomists with considerable difficulty. These difficulties are due largely to the overall morphological similarity of the species and the wide range of morphological variation within the species. Also, few collections of these species were available for study until the field work of A.M. Cleef and collaborators and J. and S. Keeley in the 1970s and 1980s. As part of continuing efforts to describe and characterize the Neotropical species of *Isoëtes* we undertook a revisionary study of this difficult group of laevigate-spored species, the *Isoëtes karstenii* A. Braun complex (Hickey, 1985).

The goals of this study were to: (1) determine the number of taxa that should be included within the *I. karstenii* complex, (2) clarify the taxonomy of the species, and (3) document intra- and interspecific morphological variation. To accomplish these goals we have collected morphological data and conducted principal components analyses of those data to infer morphologically discontinuous groups. In addition we have determined or estimated chromosome numbers for the included species. Finally, we present a taxonomic treatment that formalizes our understanding of the delimitations and relationships among these species.

TAXONOMIC HISTORY OF THE *ISOËTES KARSTENII* COMPLEX

The *Isoëtes karstenii* complex was first delimited by Hickey (1985) in a morphologically based cladistic analysis of Neotropical *Isoëtes*. It includes those

TABLE 1. Summary of the taxonomic recognition of the species historically associated with the *Isoëtes karstenii* complex. A "+" indicates the taxon was accepted as valid by the author(s); an "S" that the taxon was considered a synonym of *I. lechleri*; a "V" that the taxon was considered a variety of *I. lechleri*.

	Isoëtes lechleri	*Isoëtes karstenii*	*Isoëtes socia*	*Isoëtes colombiana*	*Isoëtes rimbachiana*	*Isoëtes palmeri*	*Isoëtes cleefii*
Mettenius (1859)	+						
Braun (1862)	+	+	+				
Baker (1880)	+	S	S				
Motelay & Vendryes (1882)	+	S	S				
Underwood (1888)	+	S	S				
Pfeiffer (1922)	+	S	S				
Weber (1922)	+	+	S				
Plamer (1929, 1932)	+	S	S	V	V		
Rodriguez (1955)	+	S	S		V		
Vareschi (1968)	+	V			V		
Gomez (1980)	+	S	S	S	S		
Fuchs-Eckert (1982)	+	+	+	+	+	+	+
Hickey (1985)	+	+	+		+	+	+

taxa with laevigate megaspores, truncate to free ala apices and corneous (darkly sclerified) leaf apices. The complex is distributed throughout the páramos of Colombia and Venezuela at elevations above 3000 m, with a single disjunct location at Mt. Chimborazo, Ecuador. Hickey (1985) included five species in this complex: *Isoëtes cleefii* H. P. Fuchs, *Isoëtes palmeri* H. P. Fuchs, *Isoëtes karstenii* A. Braun, *Isoëtes socia* A. Braun (sensu Fuchs-Eckert, 1982), and *Isoëtes rimbachiana* H. P. Fuchs. In a compendium of South American species, Fuchs-Eckert (1982) recognized eight laevigate-spored species for Venezuela, Colombia, and Ecuador and therefore may be associated with the *I. karstenii* complex: *I. socia, I. karstenii, Isoëtes steyermarkii* (nom. nud.), *I. palmeri, I. cleefii, I. rimbachiana, I. colombiana* (T. C. Palmer) H. P. Fuchs, and *Isoëtes glacialis* Asplund (including *Isoëtes arumiana* [nom. nud.] pro syn.). Fuchs-Eckert (1982), however, did not recognize these species as a distinct group. Historically these names have been treated in the literature as distinct species, or more often, as synonyms or varieties of *Isoëtes lechleri* Mett., a central Andean (southern Peru and Bolivia) species (Table 1).

MATERIALS AND METHODS

MORPHOLOGY.—Approximately 200 herbarium sheets representing about 90 discrete collection numbers of Venezuelan, Colombian, and Ecuadorian laevigate-spored *Isoëtes* were examined. We gratefully acknowledge the following herbaria for use of material: B, BM, COL, F, G, GH, MO, MU, NY, U, UC, US and VEN. Sixty-three of the collections were chosen and scored for eight quantitative morphological characters; those collections that were excluded from the analyses consisted of immature or poorly preserved material. Characters

used in the ordination and statistical analyses were: leaf number, leaf length (mm), leaf width (mm) at the mid-length, ala length/leaf length ratio, length (mm) of corneous portion of leaf apex, velum coverage (scored as a discrete character; see below), megaspore diameter (μm), and microspore length (μm). Additional characters were scored for a subset of the collections but were abandoned due to non-discriminatory age-specific correlation (e.g., corm dimensions and sporangial dimensions) or extreme variation within individuals and collections (e.g., sporangial shape and ala apex morphology). Some characters used in the analyses are also correlated with age (e.g., leaf number), or showed variability within collections (e.g., leaf length). Despite this, these characters also appeared to show group specific and discriminatory ranges of variation and were therefore retained. Terminology for morphological characters follows Hickey (1985), and for two-dimensional shapes Radford et al. (1972).

Velum coverage was scored as: (1) complete to nearly complete (> 90%), or as (2) less than half covered (25–50%). For ordination analyses, velum coverage was therefore scored as a discrete character, complete vs. incomplete.

Although microspore length and width were measured, only microspore length was chosen for the ordination and statistical analyses because most previous workers have used microspore length, rather than width, some reporting only length values. Correlation between these two characters is high for 55 collections analyzed ($r^2 = 0.877$), and either character should adequately represent the collection.

Measurements were taken only from mature plants and mature organs of those plants. Each collection, regardless of number of sheets or individuals, was considered representative of a single population unless evidence of hybridization (e.g., aborted spores) or multiple taxa within a collection (e.g., gross discontinuities in morphology) were present. Several individuals per collection were scored for each character (with the exception of microspore dimensions which were taken from a single sporangium) and a mean score for each character was calculated. The number of measurements per character differed from collection to collection. Some collections consist of only a single plant on a single herbarium sheet while others consist of numerous individuals on numerous sheets. In addition, the quality of materials varied greatly: many specimens were partially destroyed by insects, many were pressed with the substrate around the corm, and many were pressed in large clumps. As a result, some characters were difficult to obtain for all collections. Ten measurements per character per collection (25 for megaspores and microspores) were recorded whenever possible.

Two types of analyses of morphological data were conducted: (1) principal component analysis (PCA) and (2) character by character scatter plots. These analyses were performed in an attempt to reveal morphological discontinuities between groups of collections and were conducted without assigning collections to specific taxa, except for type specimens which were so designated. This was done to determine, without bias, whether or not distinct clusters of collections would be revealed which could be designated as putative morphological species and to determine which type specimen(s) would be associated

with which cluster. Once morphologically cohesive groups were recognized, descriptive statistics were calculated to document putative intraspecific variation. Multivariate and statistical analyses were performed using NTSYS version 1.80 (Rohlf, 1993) and Statmost v. 2.50 (DataMost).

CYTOLOGY.—Methods and materials for chromosome counts generally follow those of Hickey (1984). Voucher specimens for the materials from which counts were made are deposited at LOC, MU, and BM (Table 2). All specimens from which root tips were collected were grown completely submerged and potted in either fine quartz sand or a 3:1 mixture of sphagnum peat: fine quartz sand (Taylor and Luebke, 1986). Cells with mitotic figures were best obtained in root tips collected in the late morning (11:00 a.m. ± one hour). Young roots about 1 cm long were harvested and immediately placed in a saturated paradichlorobenzene solution, incubated at room temperature for 4–5 hr, fixed in Farmer's solution (3:1 95% ethanol: glacial acetic acid) and refrigerated until squashed. Root tips were hydrolyzed in 1N hydrochloric acid at 60°C for 20–30 min, neutralized in a 95% ethanol series, stained in Whittman's hematoxylin for 25 min at room temperature, rinsed in glacial acetic acid to destain, and squashed in Hoyer's mounting media. Squash slides were examined and photographed at 600× or 1000×. Color photocopies of the photographic slides were then prepared. These photocopies were placed on a light box, covered with a plain piece of white paper and the chromosomes traced onto the white paper from which counts were made. Simultaneous viewing of the photographic and/or microscope slides and the photocopy aided in interpreting slides in which chromosomes overlapped.

When live material was unavailable, we attempted to estimate chromosome number by plotting mean megaspore diameter vs. mean microspore length for taxa of known chromosome number and then plotting the mean value of spore sizes of the unknown taxa onto that plot. A strong correlation exists between spore size and chromosome number in *Isoëtes* (Cox and Hickey, 1984; Britton and Goltz, 1991). For northeastern North American taxa, Kott and Britton (1983) noted that "ploidy level can be determined with some certainty from spore measurements, even for dried herbarium material." Mean values for spore sizes of 27 taxa of known chromosome number were taken from Rury (1978), Kott and Britton (1983), Britton and Goltz (1991), Brunton et al. (1994), Taylor et al. (1993), Hickey (1994), Musselman and Knepper (1994), Brunton and Britton (1996a, 1996b), and Watanabe et al. (1996). While such correlations are unlikely to definitively identify the chromosome number of an unknown species, they may be useful in obtaining an estimate, or as in our case (see below), in ruling out potential ploidy levels.

RESULTS

MORPHOLOGY.—Included among the collections examined were type specimens (holo-, iso-, and/or paratypes) of *Isoëtes arumiana* (nom. nud.), *I. cleefii*, *I. colombiana*, *I. glacialis*, *I. karstenii*, *I. lechleri*, *I. palmeri*, *I. rimbachiana*, *I.*

TABLE 2. Chromosome counts, collection location, and voucher specimen data.

	Count	Collection Location	Voucher Location
I. karstenii	$2n = 22$	Pond, 8 km N of Pico de Aquila, Mérida, Venezuela	*Small 4 & Hickey* (MU)
	$2n = 22$	Pond, 15 km N of Pico de Aquila, Mérida, Venezuela	*Small 5 & Hickey* (MU)
	$2n = 22$	Pond, 15 km N of pico de Aquila, Mérida, Venezuela	*Small 6 & Hickey* (MU)
	$2n = 22$	Laguna Negra, Mérida, Venezuela	*Small 7 & Hickey* (MU)
	$2n = 22$	Laguna Mucubaji, Mérida, Venezuela	*Small 8 & Hickey* (MU)
	$2n = 22$	Pond, Paramo de Pisva, Municipio de Socota, Boyaca, Colombia	*Small 128 & Gonzalez* (MU, COL)
	$2n = 22$	Laguna La Pintada; Sierra Nevada del Cocuy, Boyaca, Colombia	*Small 141–143 & Gonzalez* (MU, COL)
	$2n = 22$	Laguna La Cuadrada, Sierra Nevada del Cocuy, Boyaca, Colombia	*Small 144 & Gonzalez* (MU, COL)
	$2n = 22$	Small pool near Laguna Chisaca, Cundinamarca, Colombia	*Jermy 17479* (BM)
I. palmeri	$2n = 44$	Small pool near Laguna Chisaca, Cundinamarca, Colombia	*Small 155–157 & Gonzalez* (MU, COL)
	$2n = 44$	Small pool near Laguna Chisaca, Cundinamarc, Colombia	*Jermy 17477* (BM)
	$2n = 22$	Small pool near Laguna Chisaca, Cundinamarca, Colombia	*Jermy 17482* (BM)
I. precocia	$2n = 22$	Small pool S of Laguna Larga, Cundinamarca, Colombia	*Keeley & Keeley 11032* (LOC)

TABLE 3. Eigenvectors for the first three principal components (PC) from the PCA.

Character	PC 1	PC 2	PC 3
Leaf length	0.21957	0.77593	0.21528
Leaf width	0.63580	0.16820	−0.61498
Microspore length	0.84544	−0.13050	0.28320
Megaspore diameter	0.76647	−0.05271	0.14659
% ala coverage	0.18744	−0.75048	−0.42986
Sclerified portion of leaf tip	0.04654	0.53506	−0.72654
Leaf number	0.54509	0.51899	0.12401
Velum coverage	−0.81467	0.36054	−0.04755

socia, and *I. steyermarkii* (nom. nud.). Examination of the holotypes of *I. lechleri*, *I. glacialis* and *I. socia* (all from the Andes of southern Peru or Bolivia) was performed initially to determine whether or not these taxa should be included in the subsequent analyses. Both *I. glacialis* and *I. socia* were determined to be synonyms of *I. lechleri*, which in turn was determined to be distinct from the northern Andean taxa. For a complete discussion of the variability and synonymy of the *I. lechleri* complex see Hickey (1994), but a brief discussion of our rationale for excluding these taxa follows. The synonymy of *Isoëtes socia* and *I. lechleri* has been proposed by all authors except Fuchs-Eckert (1982, 1992) since the former's publication. The type specimen of *I. socia* (*Lechler 1937b*, B!) consists of a single immature plantlet and a few isolated sporophylls. These materials were apparently removed from *Lechler 1937* (the type of *I. lechleri*) and undoubtedly represent a vegetative offset from *I. lechleri*, a species in which vegetative reproduction is common (Hickey, 1994). The type of *I. glacialis* (*Asplund 4041*, isotype B!) is but one of the many morphotypes of the highly variable *I. lechleri* and is clearly distinct, both in morphology and distribution, from *Cuatrecasas 19117*, the basis for *I. arumiana*.

Sixty-three collections were used in the PCA, including all of the type specimens except those of *Isoëtes glacialis, I. lechleri,* and *I. socia.* Eigenvectors for the first three principal components are given in Table 3; the first two principal components accounted for 36% and 23%, respectively, of the total variation. A plot of principal components one vs. two (Fig. 1) reveals six potentially distinct groups, which will be referred to in subsequent discussion by number. Clusters 1, 2 and 3 contain single collections: the type of *I. cleefii,* the "type" of *I. arumiana* (nom. nud.) and the type of *I. rimbachiana* respectively. Cluster 4 contains the type collections of *I. karstenii, I. palmeri, I. colombiana,* and *I. steyermarkii* (nom. nud.), paratypes of *I. palmeri* and *I. cleefii,* and many additional collections. Cluster 5 consists of a group of eight collections without an associated type specimen. Cluster 6 contains a group of five collections without an associated type specimen. The constituents of each of these groups were examined to determine which characters separated each group from the others and to determine whether or not these groups constitute morphologically definable taxa.

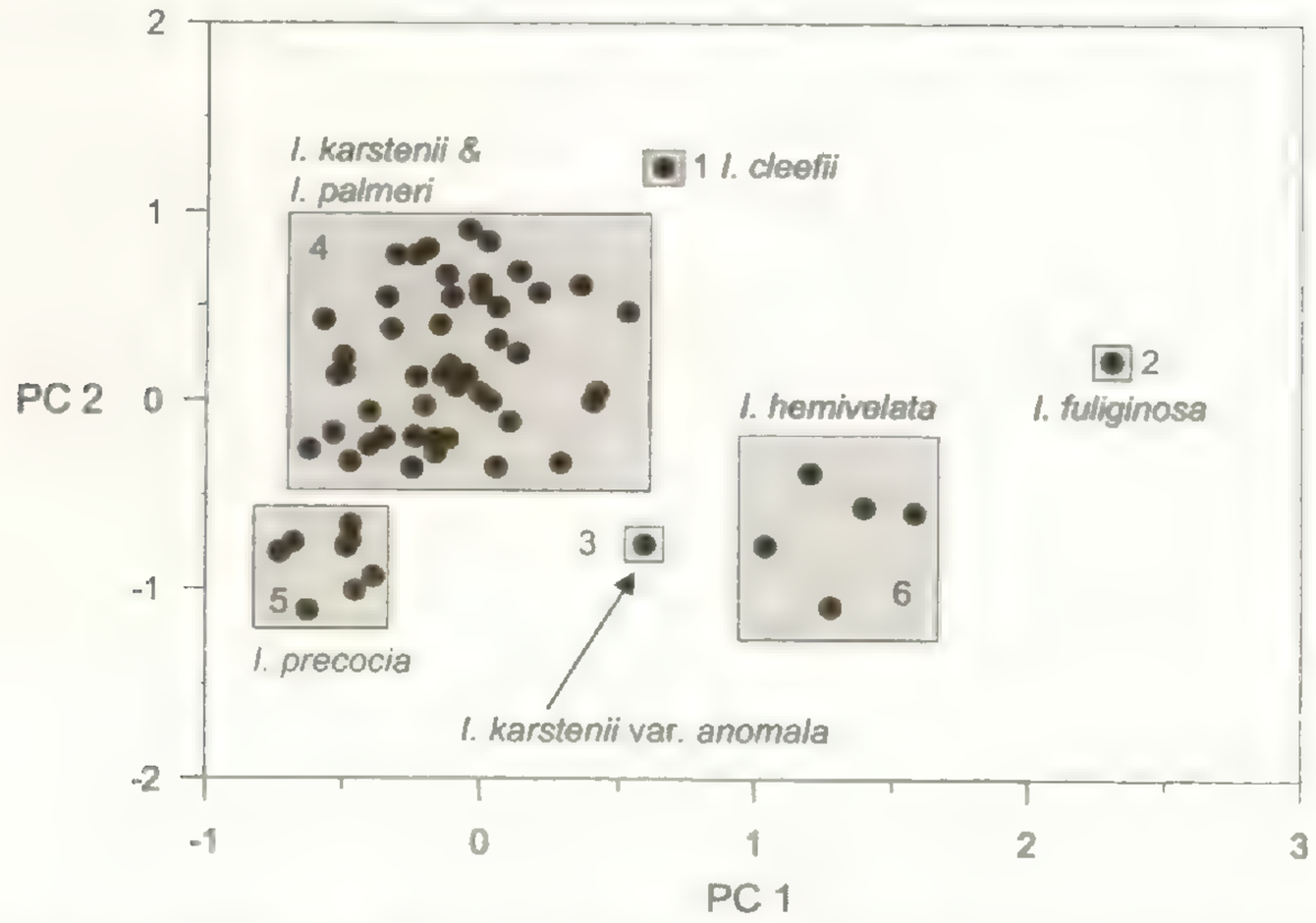


FIG. 1. Scatter plot of principal component one vs. principal component two based on morphological data. Clusters of associated specimens are indicated and numbered. 1 = *I. cleefii*; 2 = *I. fuliginosa*; 3 = *I. karstenii* var. *anomala*; 4 = *I. karstenii* + *I. palmeri*; 5 = *I. precocia*; 6 = *I. hemivelata*.

Cluster 1 (the holotype of *I. cleefii*) appears quite distinct from the remaining clusters in Fig. 1, yet examination of the characters suggests that the separation between clusters 1 and 4 is tenuous. Cluster 1 is separated from cluster 4 along principal component (PC) 2 by leaf length, leaf width and leaf number, yet character by character, the type of *I. cleefii* fell within the range of values of the individuals in cluster 4. The type collection of *I. cleefii* has longer, wider, and more numerous leaves than the average member of cluster 4. However, some collections in cluster 4 have values greater than or equal to those of the type of *I. cleefii* for some of these characters, but not for all. It appears that the combination of high values for all of these characters serve to separate the type of *I. cleefii* from the collections in cluster 4.

Cluster 2 contains the single collection, *Cuatrecasas 19117,* which is the basis of *I. arumiana* (nom. nud.). This collection is separated from the remaining collections along PC 1 by differences in velum coverage, megaspore diameter, and microspore length. In addition, the plant is larger than any other collection in this group, and the ligule is also unusually large (ca. 7 mm high) relative to the other species in this complex. This combination of character states makes this collection the most distinctive of those analyzed, and therefore we suggest that it deserves species status. To avoid further nomenclatural and taxonomic confusion caused by the invalid publication and subsequent synonymy of *I. arumiana* (nom. nud.), we propose this taxon be recognized as *Isoëtes fuliginosa* sp. nov.

Cluster 3 contains the single collection, *Rimbach 171,* the type of *Isoëtes rimbachiana* (= *I. lechleri* var. *anomala*). Differentiation of this collection from *I. karstenii* (from which it is otherwise nearly indistinguishable) by Palmer (1932) was based primarily on velum coverage, which also separates it from the collections of cluster 4 along PC 1 in our PCA. While velum coverage is

often considered to be a highly plastic character, we have found velum coverage to be uniformly complete in all collections of *I. karstenii*. Thus, the presence of an incomplete velum, along with the disjunct geographical placement of this collection at Mt. Chimborazo, Ecuador suggests that this collection represents a potentially unique biological entity and thus should be maintained as a distinct taxon. We do not feel, however, that the distinction is sufficient to warrant specific status. Because of its affinities with *I. karstenii* it should be recognized as *I. karstenii* var. *anomala* comb. nov.

Cluster 4 is a large and heterogeneous collection. Along with a large number of other collections, it contains the holotypes of *Isoëtes karstenii, I. colombiana, I. steyermarkii* (nom. nud.), and *I. palmeri,* as well as paratypes of both *I. palmeri* and *I. cleefii*. Due to this heterogeneity and the dubious separation of *I. cleefii,* we performed additional analyses on this group (see below).

Cluster 5 contains a group of eight collections without an associated type specimen. While placed close to cluster 4 (Fig. 1), this group of collections exhibits a unique set of character states that set it apart from the remaining specimens. Specifically, the PCA separates them from other specimens based on their diminutive size (leaf length, leaf width, and leaf number) at maturity, and the reduction of the corneous leaf tip. These specimens are also ecologically differentiated as they are found solely in ephemeral pools, not permanent lakes and ponds as are the other species of this group. We propose that this taxon be recognized as *Isoëtes precocia* sp. nov.

Cluster 6 contains a group of five collections without an associated type specimen. These collections are distinguished from the remaining collections along PC 1 by velum coverage, megaspore diameter, and microspore length. This group of specimens is also unique within the complex in having trigonal rather than terete leaves. Although similar to cluster 2 (*Isoëtes fuliginosa*) in velum coverage and spore dimensions, the two are easily distinguishable based on leaf cross-sectional shape, leaf length, ala length / leaf length ratio, leaf number, and the length of the sclerified portion of the leaf tip. We propose that this unique taxon be recognized as *Isoëtes hemivelata* sp. nov.

To examine the possibility that additional undetected groups existed within cluster 4 and to determine the status of *Isoëtes cleefii,* two-dimensional scatter plots of all combinations of characters were produced for those collections in clusters 1 and 4. The scatter plot of leaf length vs. leaf width at the mid-point (Fig. 2) reveals two distinct groups with different character trends: one displaying relatively long, slender leaves, the other with relatively short, thick leaves. The group with relatively long, slender leaves contains type and paratype collections of *I. palmeri* and *I. cleefii* (Fig. 2) as well as a number of additional collections. The other group (Fig. 2), with relatively short, thick leaves, contains the type collections of *I. karstenii, I. colombiana,* and *I. steyermarkii* (nom. nud.) as well as a number of additional collections. Linear regression analyses of these specimens, individually and taken together support our inferences that these two groups are distinct. If all specimens in Fig. 2 are included in a regression analysis, the $r^2 = 0.0009$ ($P = 0.85$) indicates a lack of significant correlation. If however, the two groups (above and below

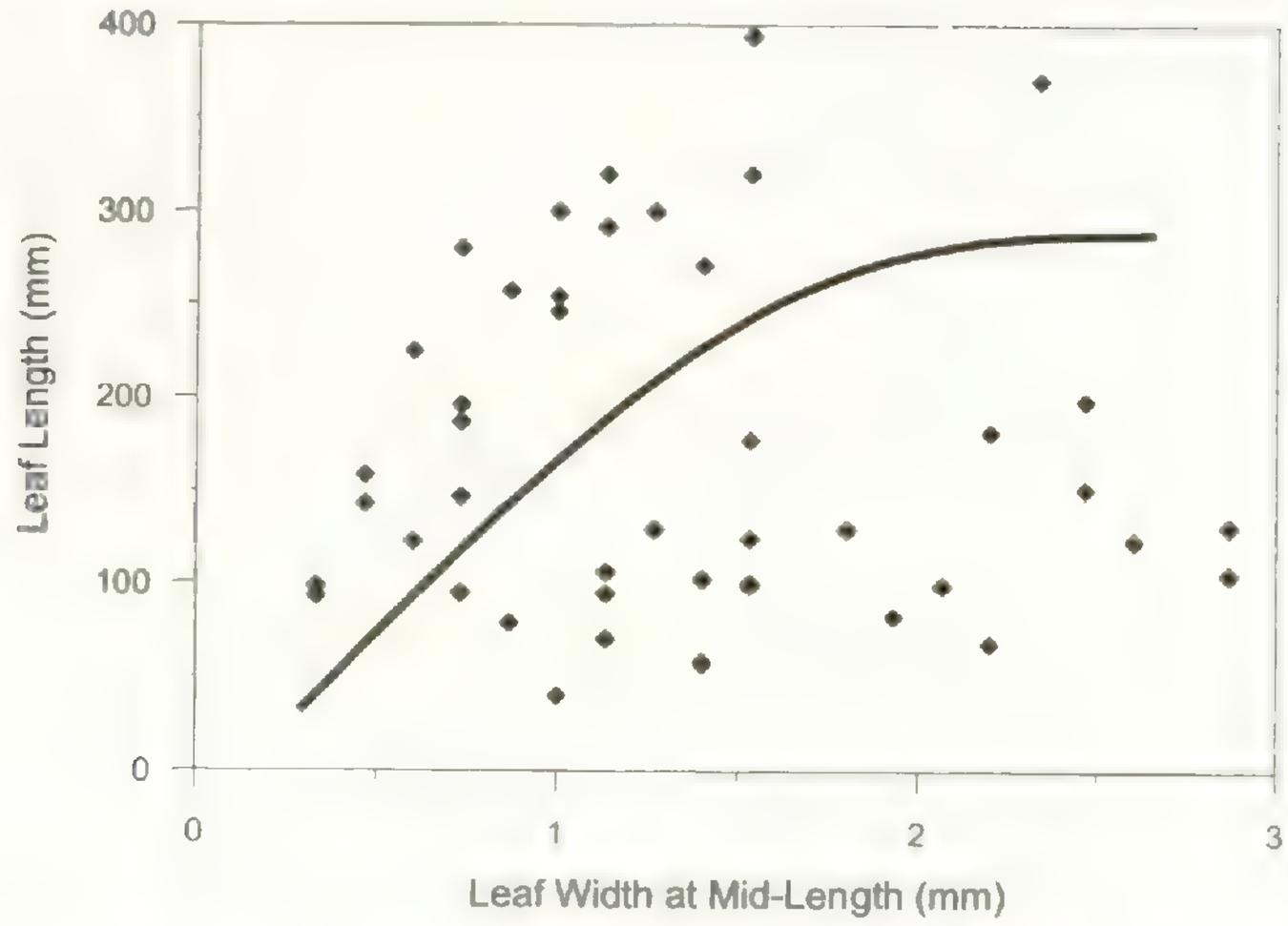


FIG. 2. Two-dimensional scatter plot of leaf length (mm) vs. leaf width (mm) for those specimens included in cluster 4 of the PCA (Fig. 1). The line divides the relatively long, narrow-leaved specimens (*I. palmeri*) from the relatively short, stout-leaved specimens (*I. karstenii*).

the line in Fig. 2) are analyzed separately, values of $r^2 = 0.70$ ($P = 1.2 \times 10^{-6}$) and $r^2 = 0.25$ ($P = 0.012$), respectively, are obtained. These values indicate that leaf length and width are significantly correlated within groups, thus lending support to our hypothesis that they are biologically distinct. The separation of these two very similar groups appears to have been obscured in the initial PCA by the inclusion of such a morphologically diverse selection of specimens. The range of variation encompassed by the initial PCA was much larger than the variation between groups within cluster 4, therefore the entire range of variation encompassed by cluster 4 was confined to a small portion of the entire PCA space. A more fine-scaled analysis was then required to detect these differences. In addition to the separation of the two groups described above, this analysis clarifies the position of the type collection of *I. cleefii* as an extreme individual along a continuum (Fig. 2).

On the basis of these analyses we delimit two additional species. The group with relatively long, slender leaves includes the type collections of *Isoëtes palmeri* and *I. cleefii*. These two taxa were published simultaneously (Fuchs-Eckert, 1981a,b), and thus neither has priority. We propose that this taxon be recognized as *I. palmeri* because the type specimen is more representative of the species overall. The group with relatively short, wide leaves includes the type collections of *I. karstenii*, *I. colombiana*, and *I. steyermarkii* (nom. nud.). The name *I. karstenii* was the first of these to be published (Braun, 1862) and therefore has priority.

In order to document the variation within these species, we calculated the mean and range of each character (Table 4). Although leaf length / leaf width at the mid-point ratio was not included as a character in the PCA (because both leaf length and leaf width were included as separate characters) it is an important character in discriminating *Isoëtes karstenii* from *I. palmeri* and was therefore included in descriptive statistics. Additionally, as chromosome num-

TABLE 4. Distinguishing character states for members of the *Isoëtes karstenii* complex. For each taxon N = the number of collections sampled. For each taxon and character the range and mean ± standard error (in parentheses) are presented.

	I. karstenii (N = 24)	*I. palmeri* (N = 22)	*I. precocia* (N = 8)	*I. hemivelata* (N = 8)	*I. fuliginosa* (N = 1)
Leaf Number	8–(13 ± 3.5)–21	7–(14 ± 4.6)–22	6–(7 ± 1.6)–11	11–(13 ± 1.8)–16	25
Leaf length (mm)	40–(115 ± 53.8)–251	93–(230 ± 90.2)–394	37–(52 ± 19.0)–94	55–(129 ± 56.7)–184	255
Leaf width at mid-length (mm)	0.9–(1.6 ± 0.4)–2.4	0.5–(1.0 ± 0.4)–1.4	0.5–(0.7 ± 0.2)–0.9	1.2–(1.9 ± 0.5)–2.4	1.9
Leaf length/leaf width ratio	36–(75 ± 31.4)–157	119–(241 ± 54.1)–350	46–(80 ± 34.5)–157	46–(64 ± 16.2)–82	134
Ala length/leaf length ratio	0.14–(0.26 ± 0.06)–0.38	0.11–(0.19 ± 0.04)–0.28	0.26–(0.30 ± 0.04)–0.36	0.28–(0.34 ± 0.05)–0.41	0.15
Corneos portion of leaf apex (mm)	0.7–(1.8 ± 0.06)–3.2	0.8–(1.4 ± 0.5)–2.4	0.1–(0.2 ± 0.1)–0.5	0.8–(1.2 ± 0.3)–1.6	0
Velum coverage (%)	>90%	>90%	>90%	35–75%	50–75%
Megaspore diameter (μm)	395–(479 ± 110.9)–507	388–(488 ± 119.9)–551	388–(442 ± 37.2)–495	513–(565 ± 49.1)- 622	600–700
Microspore length (μm)	25–(30 ± 3.7)–38	26–(31 ± 3.1)–36	27–(29 ± 1.1)–30	37–(39 ± 1.3)- 40	48
Chromosome number	$2n = 22$	$2n = 44$	$2n = 22$	$2n \geq 44$	$2n \geq 44$

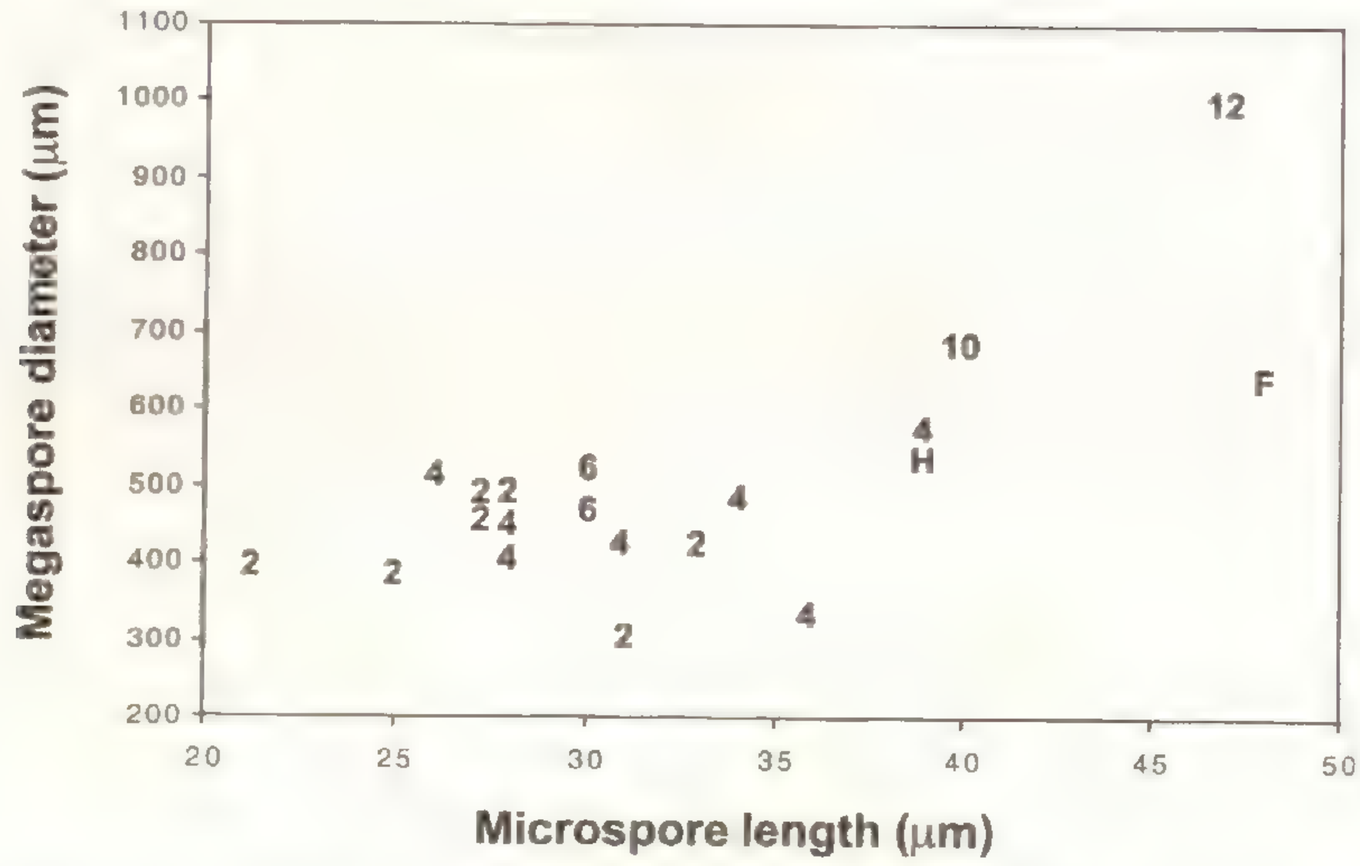


FIG. 3. Two-dimensional scatter plot of microspore length (μm) vs. megaspore diameter (μm) for species of *Isoëtes* with known chromosome numbers. Each point represents a single species with its chromosome number identified by number (e.g., "2" = 2x = 22, "4" = 4x = 44, etc.). The two unknown species are identified by letter ("H" = *I. hemivelata*; "F" = *I. fuliginosa*).

bers are important in distinguishing taxa (see below), these data are also provided in Table 4.

CYTOLOGY.—Chromosome counts were performed for *Isoëtes karstenii, I. palmeri*, and *I. precocia* (Table 2). It was found that *I. karstenii* and *I. precocia* were diploids ($2n = 22$) and *I. palmeri* was tetraploid ($2n = 44$). In addition to numerous counts of $2n = 44$ for *I. palmeri*, a single individual from one population of *I. palmeri* was found to be a triploid ($2n = 33$). This individual presumably represents a hybrid between *I. palmeri* and a diploid species, such as *I. karstenii* or *I. precocia*, both of which are sympatric with *I. palmeri* in the area of this population. These cytological data support the findings of the morphological analyses in further distinguishing *I. karstenii* and *I. precocia* from *I. palmeri*.

Live material was not available for *Isoëtes fuliginosa* or *I. hemivelata*. The relatively large size of the mega- and microspores of these taxa, however, suggest that they are most likely polyploid. We attempted to estimate the chromosome numbers of these taxa by comparing their spore sizes with taxa of known chromosome number. A scatter plot of megaspore diameter vs. microspore length was produced with individuals identified by chromosome number (Fig. 3). Individual ploidy clusters show overlap with adjacent ploidy clusters and thus it is not possible to confidently characterize the chromosome numbers of the unknown species. Despite this, these data clearly indicate that these taxa are polyploid, both being well out of the range of known diploids. *Isoëtes fuliginosa* appears to be a high-level polyploid, probably at least a hexaploid. *Isoëtes hemivelata* is more difficult to characterize because it falls out near taxa of three different ploidy levels, yet the data suggest that it is at least a tetraploid, possibly higher.

Discussion

Taxonomy and Analyses

As a result of our morphological and cytological analyses, we recognize the following taxa within the *I. karstenii* species complex: *I. fuliginosa*; *I. hemivelata*; *I. karstenii* var. *karstenii*; *I. karstenii* var. *anomala*; *I. palmeri*; and *I. precocia*. The details of this taxonomic history are given in Table 1.

The conclusions presented here are based on evaluation of morphological characters using multivariate analyses to derive distinct groups of populations. In the case of these taxa, traditional approaches using intuitive taxonomic assessments have led to undue lumping (placing all taxa in *I. lechleri*) or splitting (recognition of up to eight taxa within this complex by Fuchs-Eckert 1982). Many workers have discussed the morphological plasticity in *Isoëtes,* yet few have emphasized the fact that circumscribing that variation is imperative for understanding species delimitations (but see Kott and Britton, 1985). Evidence presented in this study supports the utility of quantitative vegetative characters in delimiting species, especially when those characters are examined using a multivariate approach.

Cytology and Speciation

Speciation in *Isoëtes* appears to occur primarily via two pathways: (1) allopatric speciation of populations of the same ploidy level, and (2) speciation via allopolyploidy (Taylor and Hickey, 1992). Cytological and morphological data suggest that both modes of speciation have occurred in the *I. karstenii* complex.

Reticulate evolution involving diploids and allopolyploids has been well documented in *Isoëtes* (Taylor et al., 1985; Hickey et al., 1989; Taylor and Hickey, 1992), yet autopolyploidy is apparently rare, having been reported only once (Rumsey et al., 1993). Allopolyploids in *Isoëtes* are generally characterized by an intermediate morphology with respect to the parental species, especially in megaspore morphology, and additive chromosome numbers and allozyme banding patterns (Taylor and Hickey, 1992). Autopolyploids would be expected to strongly resemble the parental species in all characters except chromosome number and spore size.

Because the tetraploid *Isoëtes palmeri* has completely laevigate megaspores and *Isoëtes* allopolyploids appear to always have intermediate megaspore surface morphology only those diploid taxa with laevigate megaspores should be considered as possible parents. Based on the results of this study only *I. karstenii* and/or *I. precocia* are likely to be involved.

Although conclusive evidence to address the auto- vs. allopolyploid origin of *I. palmeri* is unavailable, the data do suggest that the allopolyploid hypothesis is more plausible for several reasons. First, as mentioned, autopolyploidy is rare in *Isoëtes,* but allopolyploidy is frequent. Second, although *I. karstenii, I. precocia,* and *I. palmeri* are all morphologically similar, *I. palmeri* is intermediate for some characters (e.g., leaf width, length of corneous portion of the

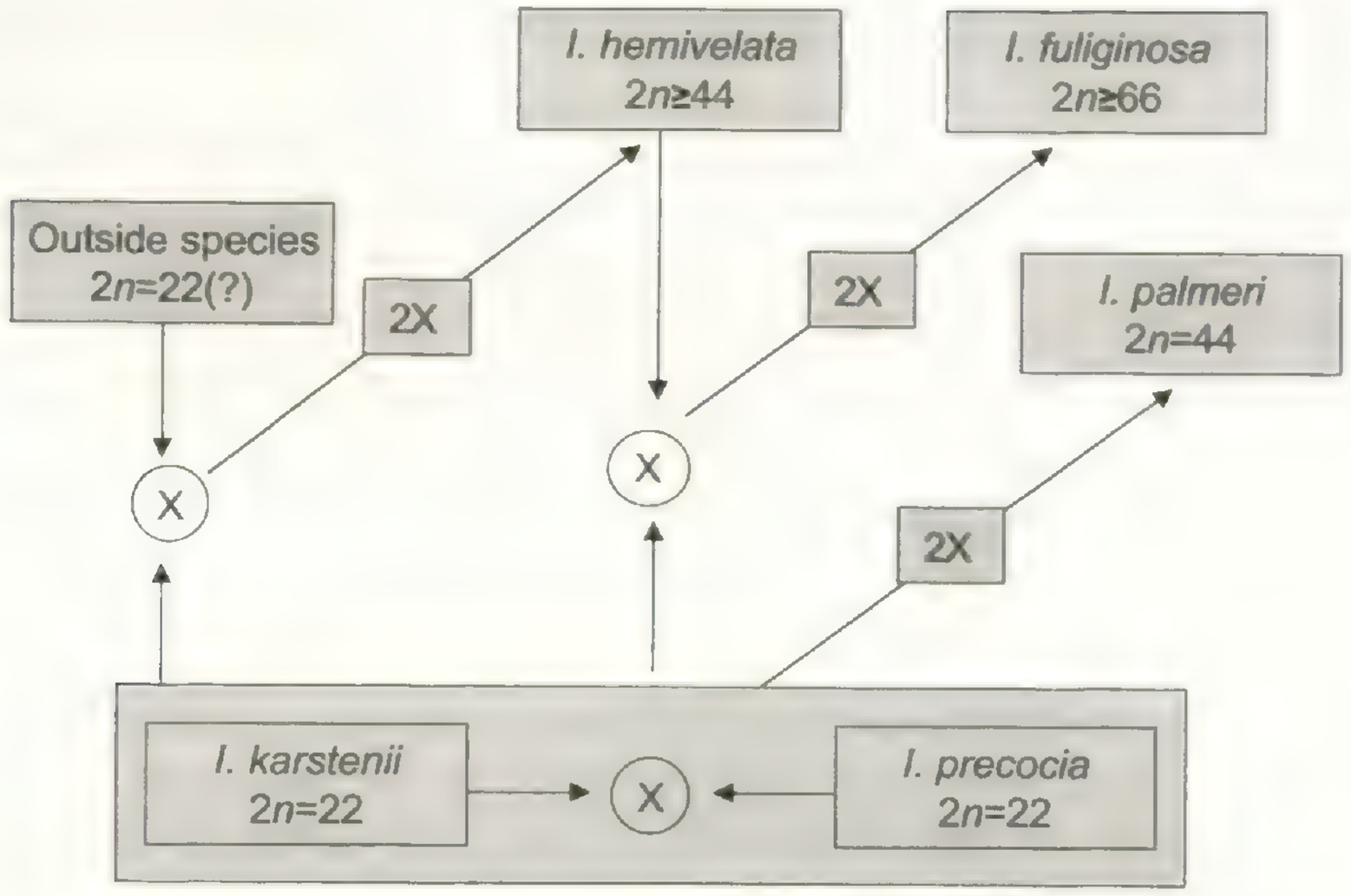


FIG. 4. Hypothesized relationships among the species of the *I. karstenii* complex. Circled ×'s represent a hybridization event; boxed 2×'s represents a chromosome doubling event.

leaf). Finally, the presence of a triploid in one of the populations of *I. palmeri* suggests that hybridization occurs between *I. palmeri* and one (or both) of the diploids. Although this does not bear directly on the origin of *I. palmeri,* it suggests that hybridization does occur in this group.

Isoëtes karstenii and *I. precocia* are both diploids and therefore have their origins in divergent (rather than reticulate) speciation. These two species exist in different habitats: *I. karstenii* is evergreen and is found in permanent lakes and ponds while *I. precocia* is ephemeral and is confined to small, seasonal pools which fill during the wet season and desiccate during the dry season. Given the phenetic resemblance and geographic sympatry of these two taxa they are probably closely related. Whether one gave rise to the other or they are each descendants of a now extinct taxon is beyond the scope of this study.

Species Relationships

Although further taxonomic and biosystematic work needs to be done, we offer a preliminary hypothesis regarding the relationships among the species in this complex (Fig. 4). The base of these relationships rest on the two diploid species, *I. karstenii* and *I. precocia.* Hybridization between these two species followed by chromosome doubling in the F_1 results in the allotetraploid, *I. palmeri.*

The origin of *I. hemivelata* is less clear, but one possibility is hybridization between a member of the *I. karstenii* complex and a species outside the complex, possibly *I. andina* or one of several undescribed Colombian or Ecuadorian species (Hickey, unpublished data). The evidence to support this includes: *I. hemivelata*'s possession of character states derived from the *I. karstenii* com-

plex and from the *I. andina* complex, along with its undoubtedly polyploid nature.

The origin of *I. fuliginosa* possibly occurred by hybridization between *I. hemivelata* and another member of the *I. karstenii* complex. *Isoëtes fuliginosa* is clearly polyploid, shares the character state of reduced velum coverage with *I. hemivelata,* and is larger than the other members of the *I. karstenii* complex, but in other respects maintains the characters of the complex.

Taxonomic Treatment

Isoëtes karstenii Complex

Corm globose to horizontally elongate, two lobed (rarely three), size variable; dichotomously branching roots arising from the circumbasal fossa(e). Leaves variable in number, length, and width, flexuous or stiff; alae chartaceous, brown to black, extending upward to 50% of total leaf length, ala apices attenuate, acute, truncate or free; subula terete to trigonal, olive green to brown; leaf apices corneous, typically spatulate. Scale leaves, phyllopodia, stomates and peripheral fibrous bundles not seen. Sporangia (ob)ovate to elliptic, tan, concolorous to spotted, basal. Velum incomplete to complete. Ligule deltate to trullate. Labium absent or reduced to a small transverse ridge on the lower lip of the foveola. Megaspores white, 350–700 μm in diameter, laevigate on both proximal and distal faces. Microspores cocoa brown, 25–55 μm in length, echinate. Vegetative reproduction not observed.

As discussed previously, these taxa have often been placed in synonymy with, or regarded as varieties of, *I. lechleri.* They can be distinguished from that species, however, on the basis of ala length/leaf length ratio (up to 50% in the *I. karstenii* complex vs. 50–75% in *I. lechleri*), ala apex morphology (attenuate to free in the *I. karstenii* complex vs. always attenuate in *I. lechleri*), vegetative reproduction (common in *I. lechleri* vs. absent in the *I. karstenii* complex), and distribution (*I. karstenii* complex in Ecuador, Colombia and Venezuela vs. *I. lechleri* in southern Peru and Bolivia). Additionally, the only member of the *I. karstenii* complex which strongly resembles *I. lechleri* is *I. karstenii.* These two taxa can be further differentiated on the basis of chromosome number: *I. karstenii* has $2n = 22$ and *I. lechleri* has $2n = 44$ (Hickey, 1994).

Key to Species of the *I. karstenii* Complex

1. Velum covering 25–75% of the sporangium
   2. Alae extending 10–20% up the leaf length; leaves 150–310 mm long; velum covering 50–75% of the sporangium; microspores 45–55 μm long; megaspores 600–700 μm in diameter ..... 1. *I. fuliginosa*
   2. Alae extending 25–45% up the leaf length; leaves 40–200 mm long; velum covering 30–50% of the sporangium; microspores 25–45 μm long; megaspores 300–650 μm in diameter
      3. Megaspores 300–450 μm in diameter; microspores 30–40 μm long; subula terete ..... 4b *I. karstenii* var. *anomala*

3. Megaspores 500–650 μm in diameter; microspores 35–45 μm long; subula trigonal ..... 2. *I. hemivelata*

1. Velum covering >90% of the sporangium
   4. Leaf apices not or only slightly sclerified (the sclerified part < 0.5 mm long); leaves less than 100 mm long, less than 1.0 mm wide at the middle; plants seasonally ephemeral ..... 3. *I. precocia*
   4. Leaf apices distinctly sclerified (the sclerified part 0.7–3.2 mm long); leaves 40–400 mm long, 0.5–2.4 mm wide at the middle; plants seasonally persistent
      5. Leaves 25–200 mm long and 1.0–2.5 mm wide with a leaf length/leaf width ratio of 30–160; $2n = 22$ ..... 4a. *I. karstenii* var. *karstenii*
      5. Leaves 100–400 mm long and 0.5–1.5 mm wide with a leaf length/leaf width ratio of 110–350; $2n = 44$ ..... 5. *I. palmeri*

1. **Isoëtes fuliginosa** R. L. Small and Hickey, **sp. nov.** (Fig. 5–7; 14–15).

Type: "Colombia. Departamento del Cauca: Cordillera Central, Lagunilla de las Casitas, 3700 m alt., 3 Dec 1944" *Cuatrecasas 19117* (Holotype: F!; Isotypes: COL!, F!, G-2!, GH!, MO!, MU!, US!).

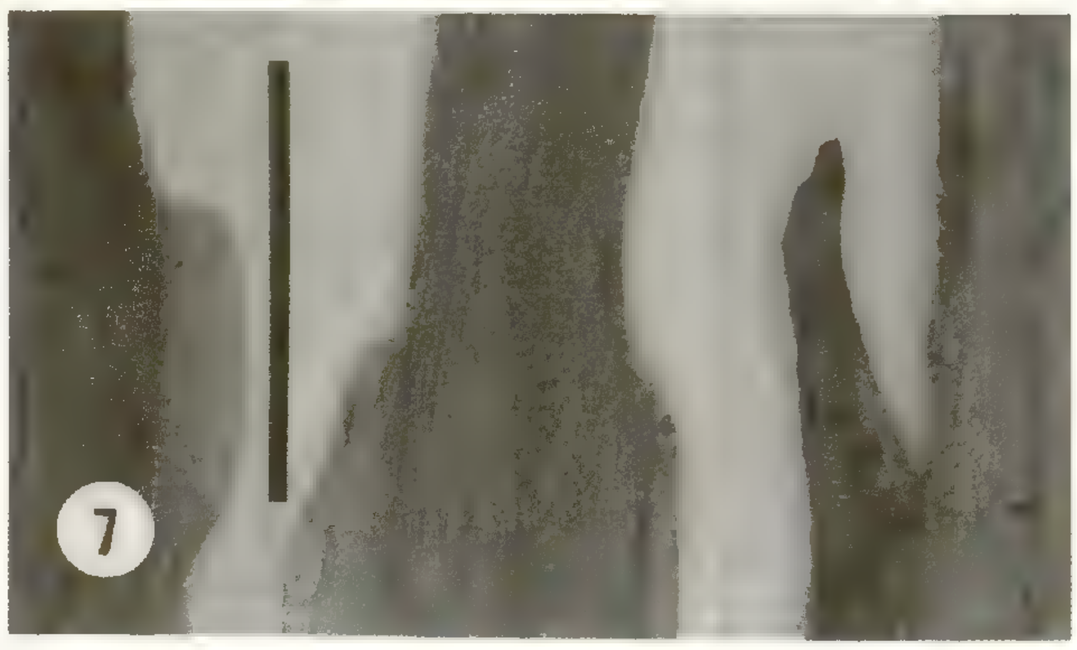
Isoëtes arumiana Fuchs-Eckert, nom. nud. Proc. Kon. Ned. Akad. Wetensch. C. 85(2):259. 1982. Based on *Cuatrecasas 19117* (US!)

Ob statura magna, velo incompleto et megasporis grandibus inter omnes *Isoëtes* species ab Andibus septentrionalibus peculiaris; *Isoëtes karstenii* A. Braun tangit, ob megasporis laevibus et ob apicibus aliis truncatis versus liberis.

Corm horizontally elongate, two-lobed, 9–10 (x = 9.7) mm high, 24–25 (x = 24.7) mm wide; dichotomously branching roots arising from the circumbasal fossa. Leaves 18–34, flexuous, to 310 mm long, 8–12 mm wide at base, 1.5–2.5 mm wide at mid-length, leaf length/width ratio 134; alae chartaceous, brown to blackened, extending 11–17% of total leaf length, apices acute to truncate; subula terete in cross-section, olive green, apex obtuse, not or only slightly corneous at the tip; peripheral fibrous bundles, stomates, scale leaves and phyllopodia absent. Sporangia obovate to ovate, basal, unpigmented, 5.5–7.8 (x = 7.2) mm long, 4.5–5.8 (x = 5.2) mm wide. Velum incomplete, covering 50–75% of the sporangial surface. Ligule deltate and auriculate, ca. 7 mm high, 5 mm wide. Labium absent or reduced to an extremely small transverse ridge on lower lip of the foveola. Megaspores immature, but ca. 600–700 μm in diameter, laevigate on both proximal and distal faces. Microspores cocoa brown, 45–55 (x = 48.2) μm in length, 30–40 (x = 35.4) μm wide, echinate. Chromosome number unknown.

Isoëtes fuliginosa is known only from the type collection which was taken from a small lake or pond ("lagunilla") at 3700 m altitude in the Cordillera Central, Departamento Cauca, Colombia (Fig. 8). This collection was made in December and contains both microspores and megaspores; however, the megaspores are immature. Further collections are necessary to characterize the phenology and full range of morphological variation of this species.

Despite the immaturity of this collection, *Isoëtes fuliginosa* is distinct from



FIGS. 5–7. *Isoëtes fuliginosa* (*Cuatrecasas 19117*). FIG. 5. Habit view showing reduced alae (F #1351470). Bar = 5 cm. FIG. 6. Microphyll base showing reduced alae and partial velum (US). Bar = 1 cm. FIG. 7. Variation in ala apices: truncate, acute and free respectively (F # 1351471). Bar = 5 mm.

other species of *Isoëtes* and can be recognized by its incomplete velum, large growth habit and large mega- and microspores. It is clearly related to the other members of the *I. karstenii* complex given its laevigate megaspores and truncate to free ala apices, although the incomplete velum and apparently high ploidy level indicate that an outside influence is also present. The results of the spore analyses show that *I. fuliginosa* is polyploid (probably hexaploid or

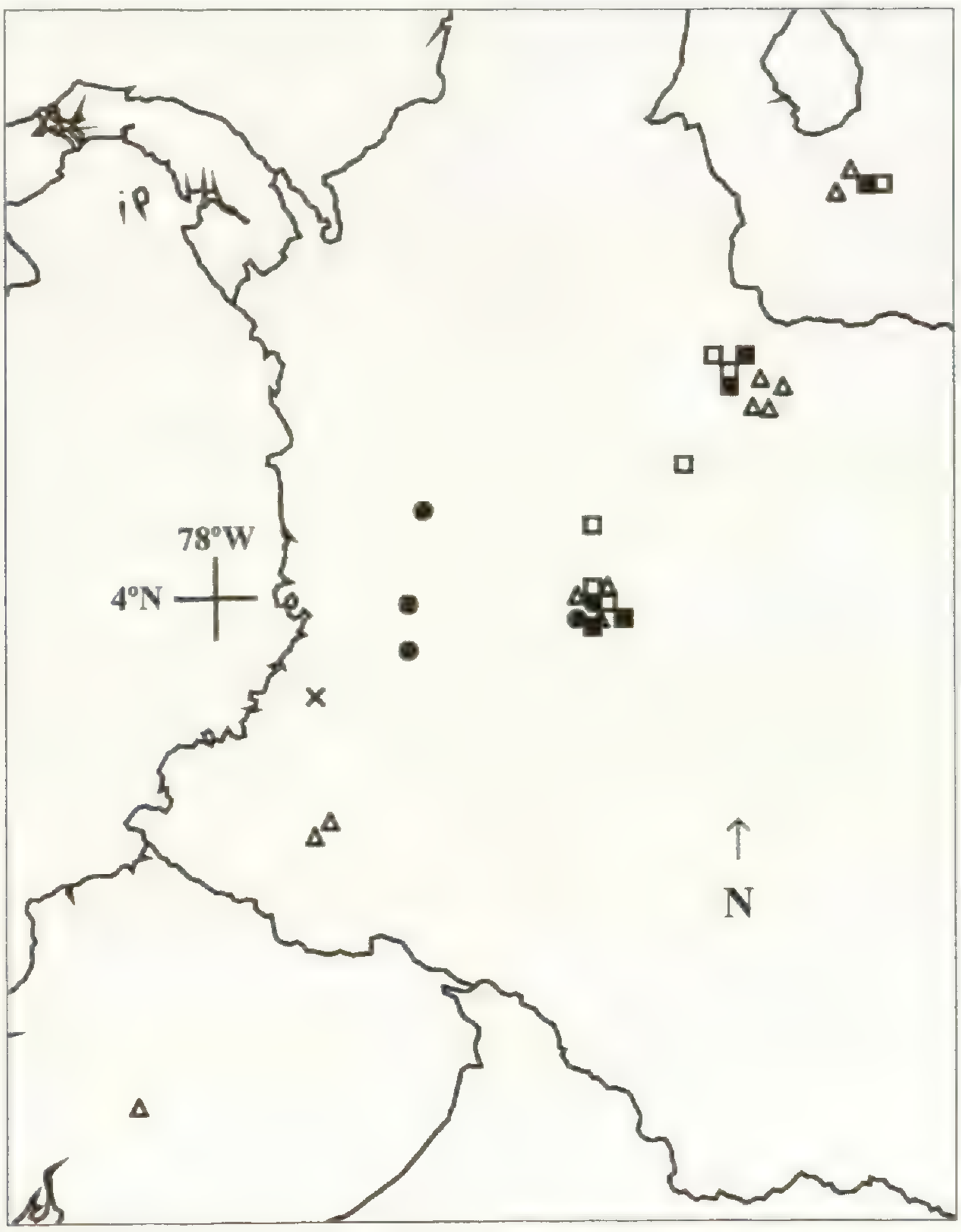


FIG. 8. Distributions of the species of the *I. karstenii* complex. Open triangles = *I. karstenii*, open squares = *I. precocia*, filled squares = *I. palmeri*, filled circles = *I. hemivelata*, and the X = *I. fuliginosa*.

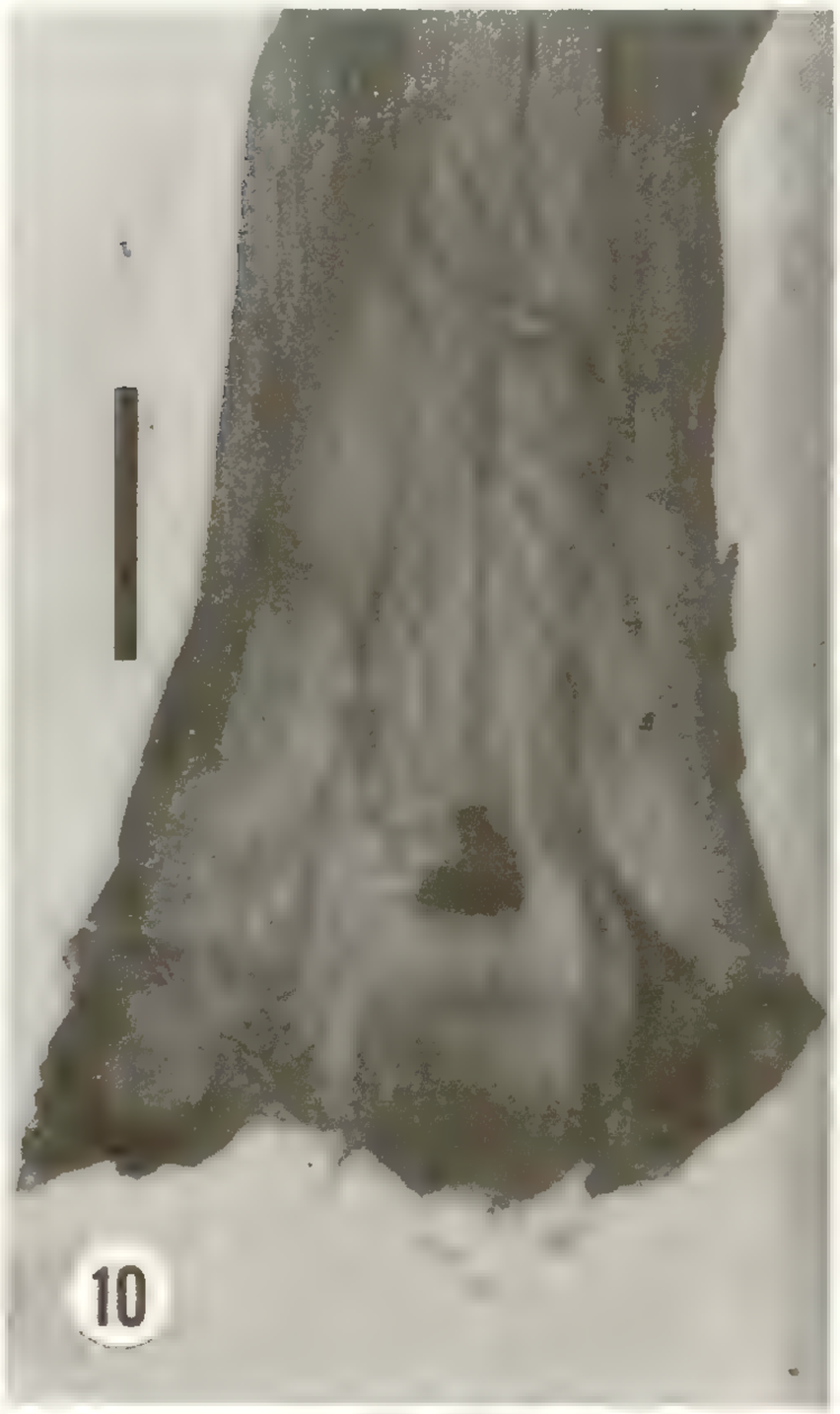
higher) and therefore is likely an allopolyploid derivative of other nothospecies.

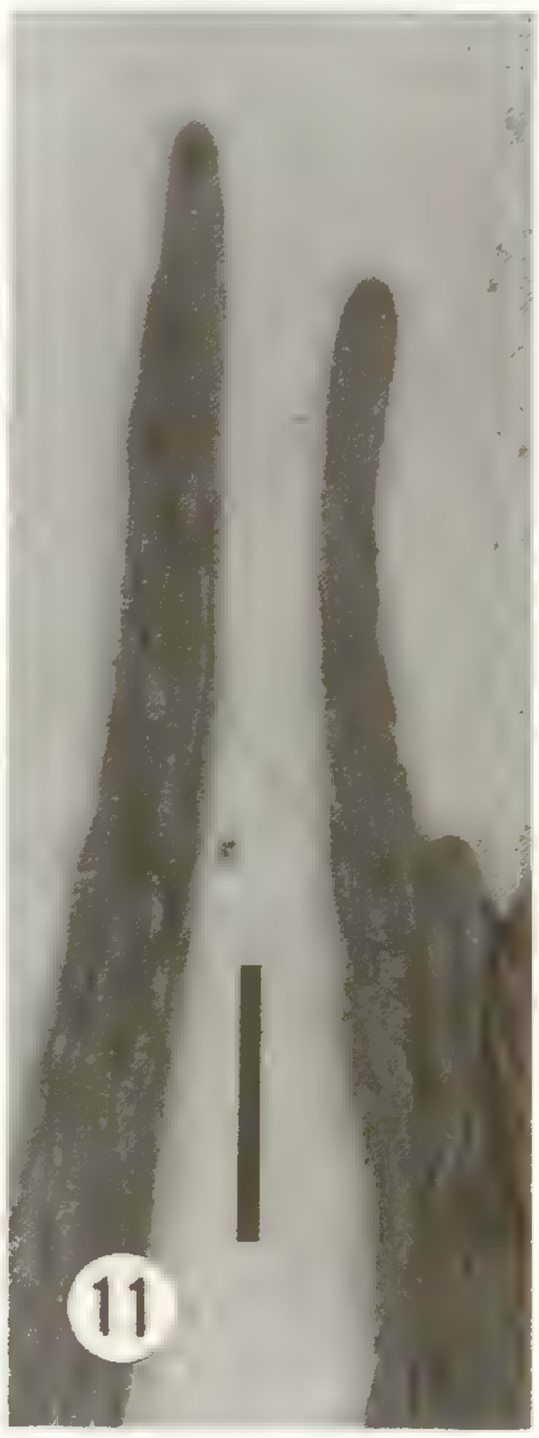
2. **Isoëtes hemivelata** R.L. Small and Hickey, **sp. nov.** (Fig. 9–11, 16–17)

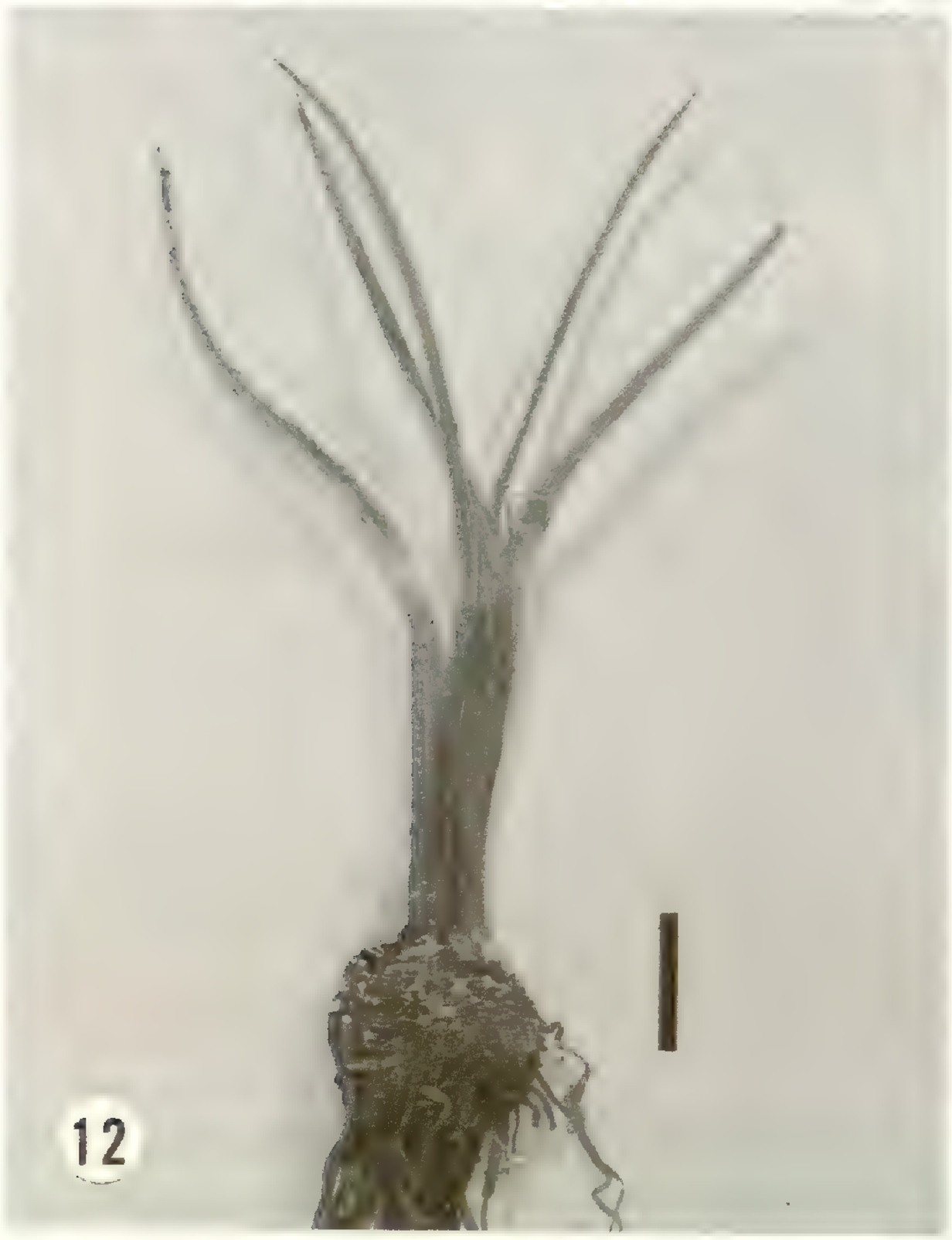
Type: "Colombia, Departamento del Valle, Cordillera Central, vertiente occidental; cabeceras de los ríos Tulua y Bugalagrande: Páramo de Las Vegas, alt 3600–3800 m, 22 March 1946" *Cuatrecasas 20301* (Holotype: F!; Isotypes: M!, NY!, UC!, US!)

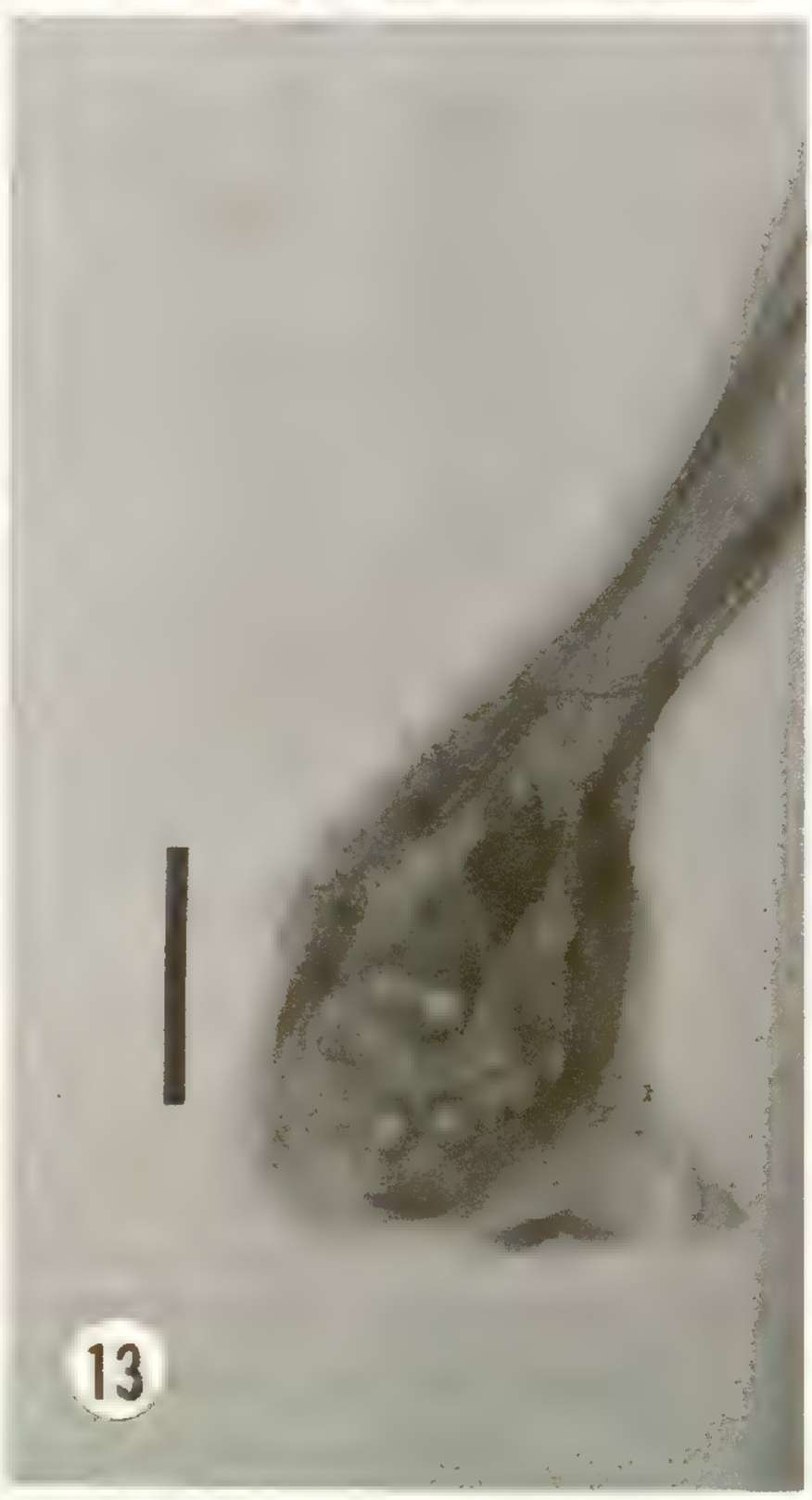
Haec species ab Sectione Isoete karstenii praeclare distinguitur subulis trigonis et velis 30–50% obtegentibus.

Corm globose, two-lobed, 4–12 (x = 6.5) mm high, 6–11 (x = 8.1) mm wide; dichotomously branching roots arising from the circumbasal fossa. Leaves 10–25, stiffly erect, to 200 mm long, 4–6 mm wide at base, 1.2–2.4 mm wide at mid-length, leaf length/width ratio 45–82 (x = 64); alae chartaceous, dark brown to black, extending 28–51% (x = 37%) of leaf length, apices acute to

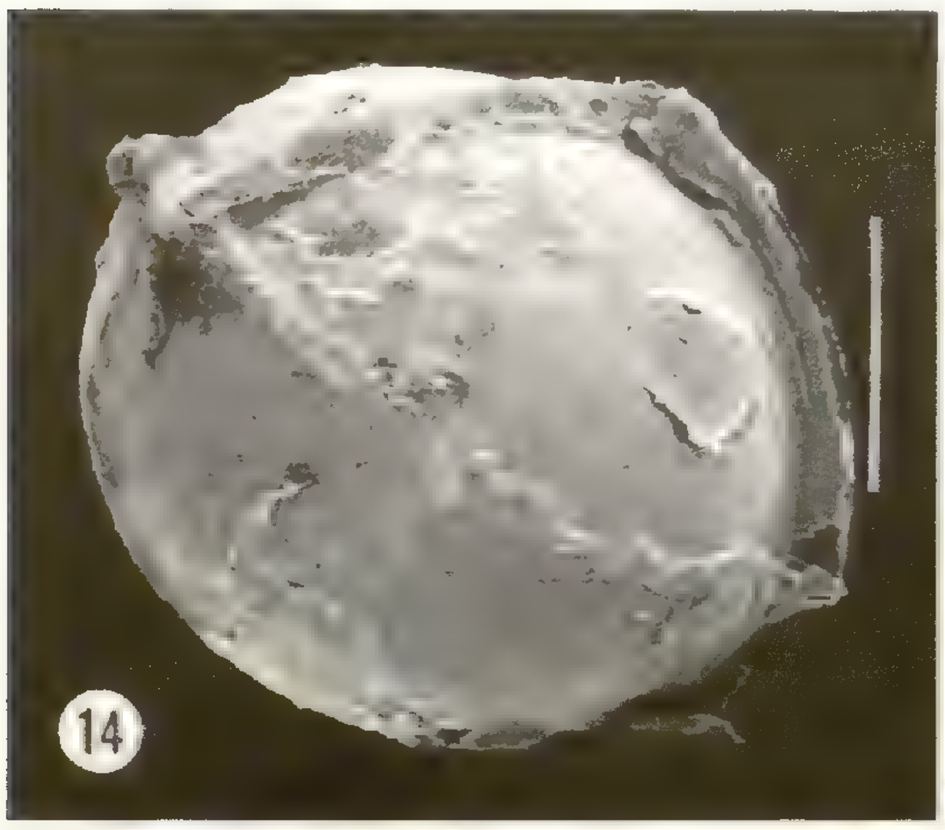


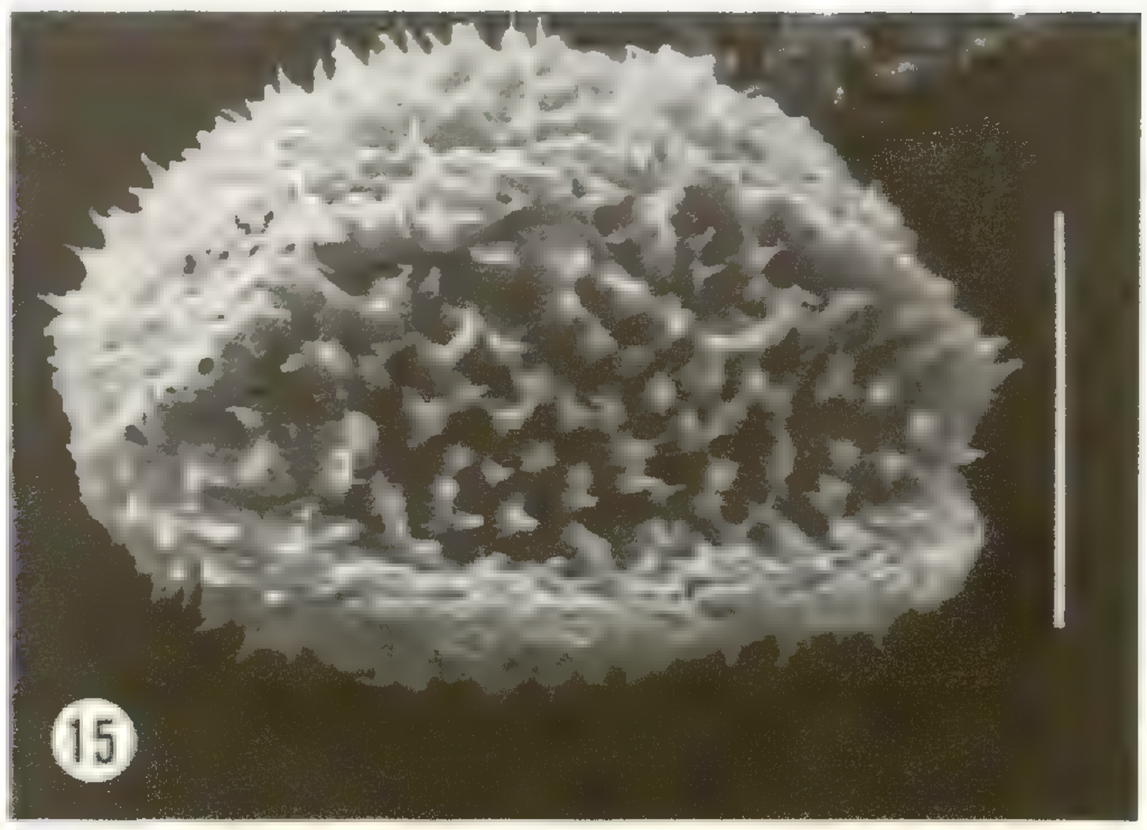


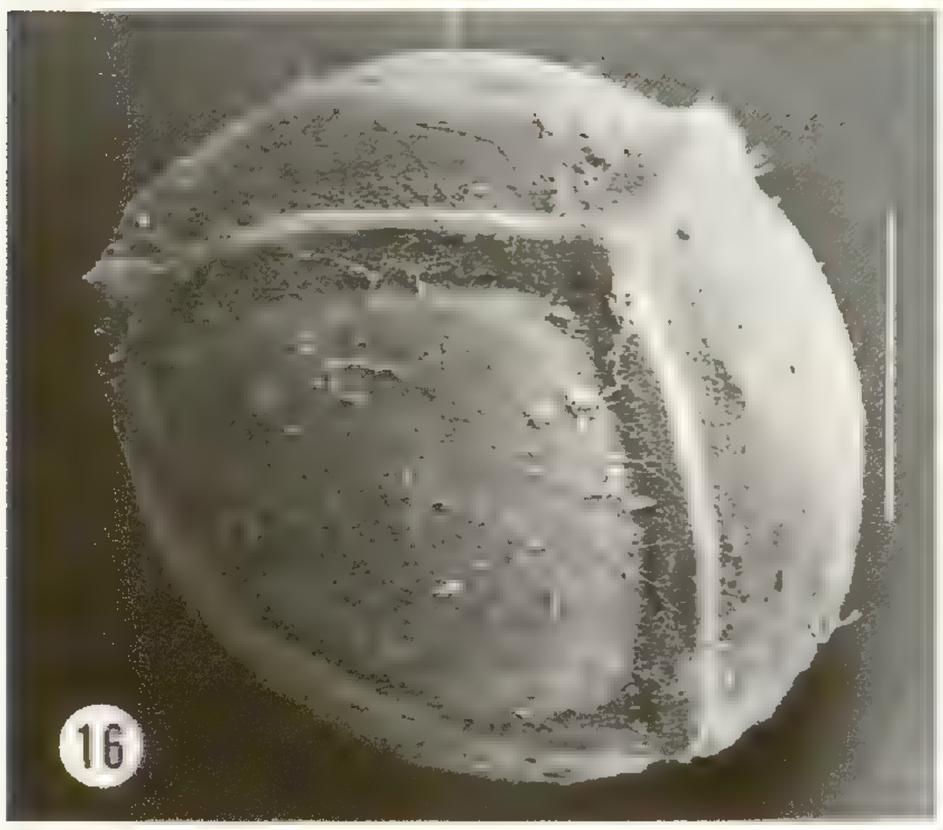


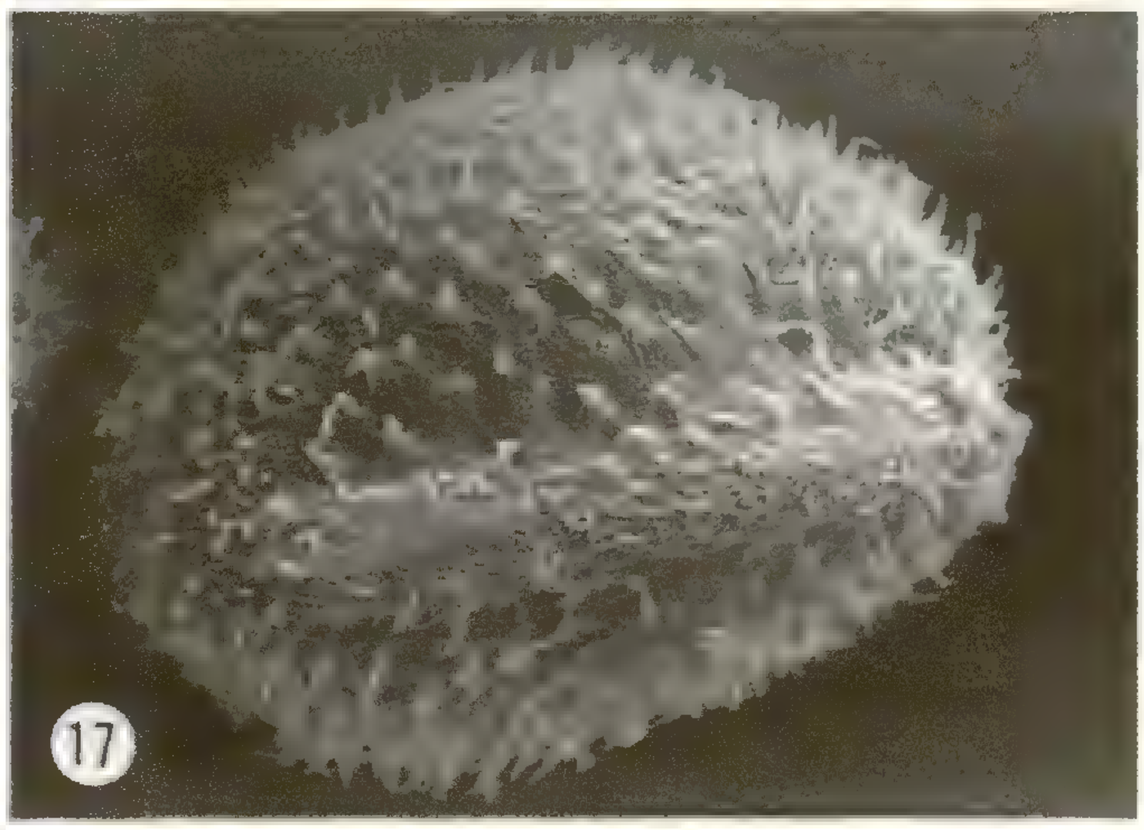


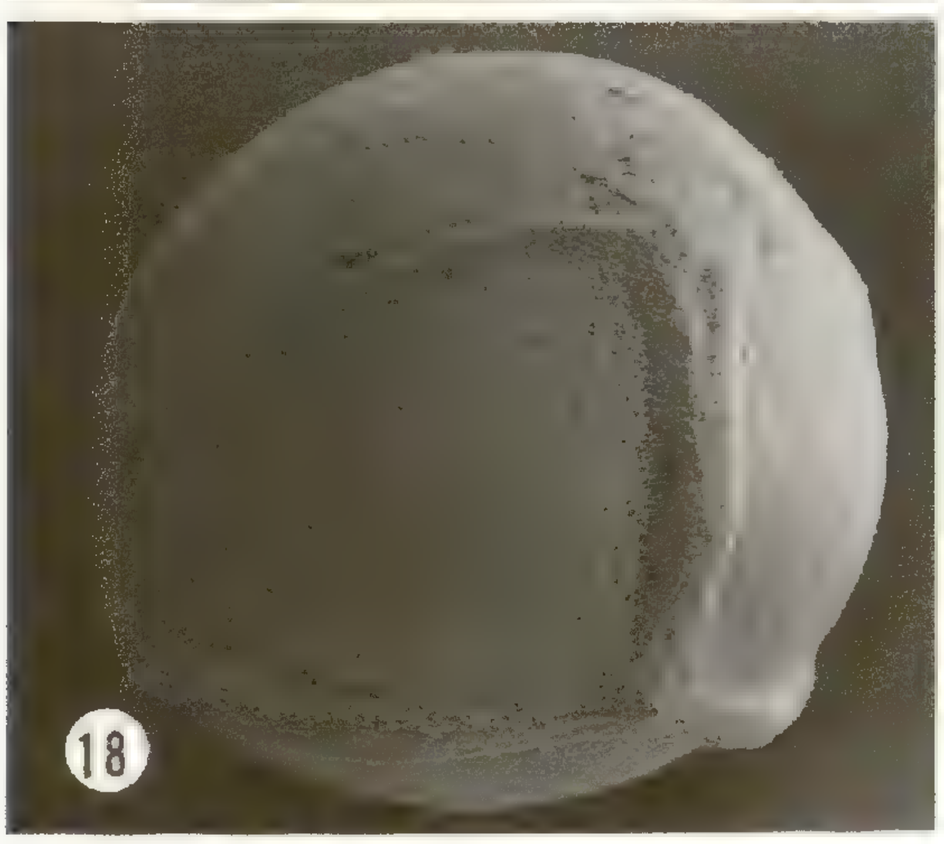
FIGS. 9–13. *Isoëtes hemivelata* (*Cuatrecasas 20301*, US) and *I. precocia* (*Oberwinkler & Oberwinkler 12968*, M). FIG. 9. Habit view of several plants of *I. hemivelata*. Bar = 2 cm. FIG. 10. Leaf base of *I. hemivelata* showing partial velum coverage. Bar = 2 mm. FIG. 11. Subula apices of *I. hemivelata* showing black, corneous apices. Bar = 2 mm. FIG. 12. Habit view of a mature fertile specimen of *I. precocia*. Bar = 1 cm. FIG. 13. Leaf base of *I. precocia* showing a well filled megasporangium with a complete velum and the narrow, parallel alae. Bar = 2 mm.

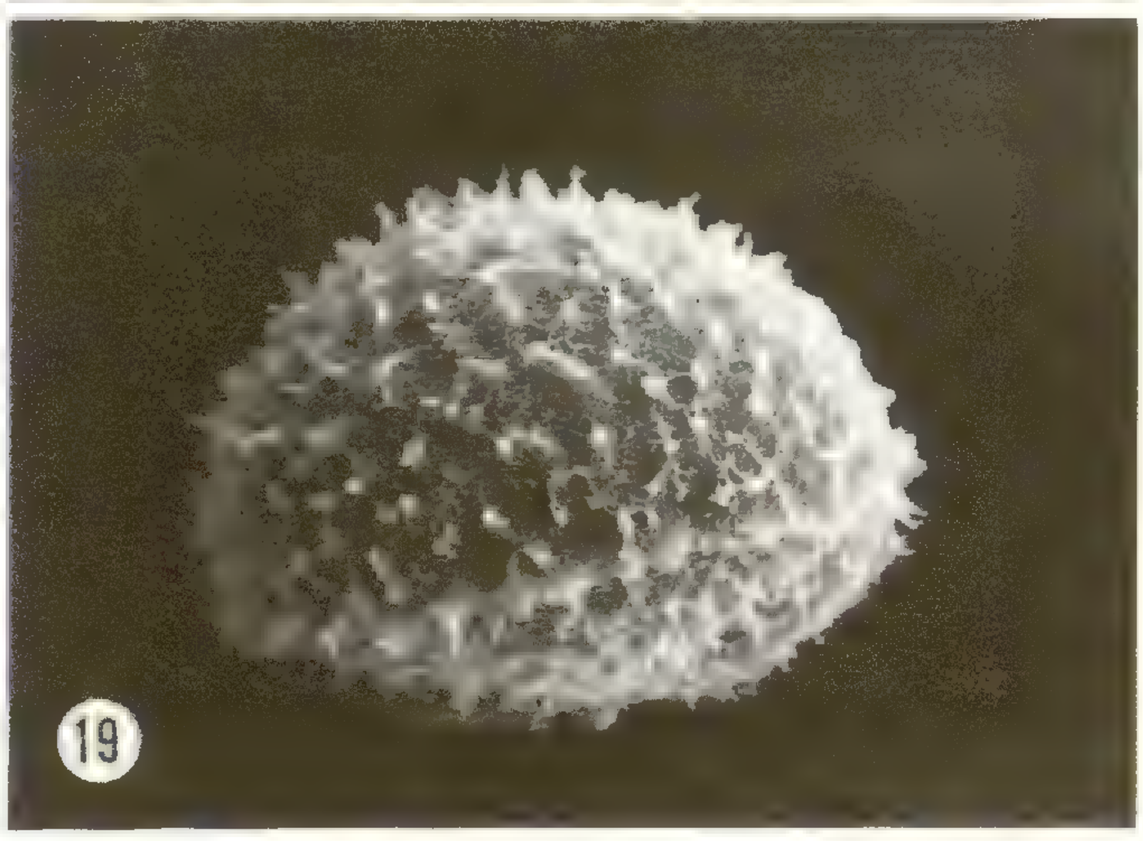












FIGS. 14–19. *Isoëtes fuliginosa, I. hemivelata, and I. precocia*. FIGS. 14–15—SEM of spores of *Isoëtes fuliginosa* (*Cuatrecasas 19117,* US). FIG. 14. Equatorial view of immature megaspore. Bar = 200 μm. FIG. 15. Proximal view of microspore showing one of the two proximal surfaces. Bar = 20 μm. FIGS. 16–17—SEM of spores of *I. hemivelata* (*Cuatrecasas 20301,* F). Fig. 16. Proximal view of megaspore. Scale as in Fig. 14. Fig. 17. Proximal view of microspore. Scale as in Fig. 15. FIGS. 18–19—SEM of spores of *I. precocia* (*Oberwinkler & Oberwinkler 12968,* M). Fig. 18. Near proximal view of megaspore. Scale as in Fig. 14. Fig. 19. Equatorial view of microspore. Scale as in Fig. 15.

free; subula ± trigonal, olive green; leaf apices blunt, often spatulate, distal 0.75–1.75 mm of apex corneous; peripheral fibrous bundles, stomates, scale leaves and phyllopodia absent. Sporangia elliptic to obovate, basal, spotted, 1.5–3.2 mm long, 1–2 mm wide. Velum incomplete, covering 30–50 % of the sporangial surface. Ligule shallowly deltate to trullate, to 1.5 mm long, to 2 mm wide. Labium absent or reduced to a transverse ridge on the lower lip of the foveola. Megaspores white, 500–650 (x = 560) μm in diameter, laevigate on both proximal and distal faces. Microspores cocoa brown, 35–40 (x = 38.5) μm long, 25–30 (x = 27.6) μm wide, echinate. Chromosome number unknown.

Isoëtes hemivelata grows as a submerged aquatic in permanent lakes and ponds (occasionally streams) between 3300–4300 m. It is distributed throughout the central and southern Departamentos of the Colombian Andes including Meta, Risaralda, Huila, Cauca and Valle (Fig. 8).

Although known from only a few collections, those included in this study were made in January, March, August and October and all contained both mega- and microspores. This suggests that this species is evergreen (as are most Andean *Isoëtes*) and produces both mega- and microspores throughout the year.

Isoëtes hemivelata is one of the most distinctive elements within the *I. karstenii* complex given its trigonal subula and incomplete velum. It can be distinguished from *I. karstenii, I. palmeri,* and *I. precocia* by these characters and its larger spores. It can be distinguished from *I. fuliginosa* by its trigonal subula, smaller spores, and greater ala length / leaf length ratio.

Analyses of spore size suggest that *I. hemivelata* is polyploid. The characters it shares with the *I. karstenii* complex indicate that one of the parental species comes from this complex; however, the trigonal subula and reduced velum coverage must have come from a source outside this complex. This suggests hybridization and polyploidization. One likely parent is *I. andina* Spruce ex Hook., a sympatric species with strongly trigonal leaves and an incomplete velum. One collection seen by us (*Cuatrecasas 19058* F!, GH!, US!) apparently represents a collection of hybrids between a member of the *I. karstenii* complex and *I. andina.* This collection is highly variable for most characters and spore abortion is evident. While most megaspores of this collection are slightly to distinctly papillate, some are almost laevigate and all individuals of the collection have ± trigonal leaves. Chromosome doubling in a hybrid such as this, or backcrossing to the *I. karstenii* complex parent could feasibly result in a taxon which strongly resembles *I. hemivelata.*

Additional specimens examined:

COLOMBIA. **Departamentos Huila/Cauca:** Macizo Colombiano; páramo de Las Papas, Cerros y alrededores de la laguna La Magdalena, alt. 3530 m, 16 Oct 1958, *Idrobo, et al. 3307* (COL, NY). **Departamento Meta:** Páramo de Sumapaz, Hoya Sitiales, Laguna La Primavera y alrededores, alt. 3510 m, 9 Jan 1973, *Cleef 7555* (COL, U); Páramo de Sumapaz, Hoya El Nevado, Laguna La Guitarra y alrededores, alt. 3425 m, 23 Jan 1973, *Cleef 8268* (COL, U). **Departamento Risaralda:** Cordillera Central; Municipie de Pereira; parque de los Nevados, alt 4250 m, *Jaramillo, et al. 5700* (COL, U). **Departamento del Valle:** Páramo Pan de Azucar, alt 3300–3700 m, 23 Aug 1968, *Espinal & Ramos 2471* (COL).

3. **Isoëtes precocia** R.L. Small and Hickey, **sp. nov.** (Fig. 12–13, 18–19)

Type: "Venezuela: Anden, Estado Mérida: Sierra de St. Domingo, Páramo de Mucubaji, Weg zur Laguna Negra, an stark vernäßten Stellen, ± 3500 m. 4. 10. 1968." *B & F Oberwinkler 12968* (Holotype: M!; Isotype: VEN!).

Isoëtes socia sensu Fuchs-Eckert 1982 *pro parte,* Proc. Kon. Ned. Akad. Wetensch. C. 85(2): 256 1982. *non* A. Braun.

Species nova *I. karstenii* proxima, cujus megasporas laeves et velum completum habet; differt staturo minore, apicibus foliis brevissimis corneis et habitu ephemero.

Corm globose to horizontally elongate, two lobed, 1.8–2.4 mm (x = 2.1) high, 2.5–4.6 mm (x = 3.6) wide; dichotomously branching roots arising from the circumbasal fossa. Leaves 6–11, flexuous, to 63 mm long, to 4 mm wide at base, 0.5 to 0.9 mm wide at mid-length, leaf length / width ratio 45–157 (x = 80); alae chartaceous, caramel-colored, extending 26–36% of total leaf length, apices acute to truncate; subula essentially terete in cross-section, pale green, apices blunt, corneous only at 0.1–0.2 mm of the tip; peripheral fibrous bundles, stomates, scale leaves and phyllopodia absent. Sporangia elliptic to circular, basal, unpigmented, 1.6–2.7 mm (x = 2.1) long, 1.2–2.1 mm (x = 1.7) wide. Velum complete, covering 100% of the sporangial surface (rarely slightly less). Ligule ephemeral, not seen. Labium absent or reduced to an extremely small transverse ridge of tissue on lower lip of the foveola. Megaspores white, lustrous, 388–495 (x = 437) μm in diameter, laevigate on both proximal and distal faces. Microspores cocoa brown, 27–30 (x = 29) μm in length 18.9–23.3 (x = 20.5) μm wide, echinate. Chromosome number: $2n = 22$.

Isoëtes precocia grows submerged in shallow, temporary pools at 3300–4400 m. It is currently known from Estado Mérida, Venezuela and Departamentos Boyaca, Cundinamarca and Meta in Colombia (Fig. 8). Although only eleven collections of this species have been found among the specimens examined, it is probably a common plant of seasonal pools but has not been collected due to its diminutive size and ephemeral nature. We visited the type locality in March of 1992, yet were unable to find this species because the pools were dry. Collections examined in this study were made in March, April, July, October, November and December, and all contained mega- and microspores.

Fuchs-Eckert (1982) recognized that a taxon resembling the type of *I. socia* existed, but failed to differentiate between the vegetative plantlets of *I. lechleri* in Peru and Bolivia and the distinct species (*I. precocia*) of Colombia and Venezuela. Hickey (1985) recognized that a distinct species existed, but did not study the associated specimens and based his acceptance of the name *I. socia* on the work of Fuchs-Eckert (1982). Hickey (1994) has subsequently placed *I. socia* in synonymy with *I. lechleri.*

Additional specimens examined:

COLOMBIA. **Departamento Boyacá:** Sierra Nevada del Cocuy, Alto Valle Lagunillas, 1.5 km NE de la Laguna Pintada, 4390 m, 30 Nov 1972, *Cleef 5704* (U); Sierra Nevada del Cocuy, Boqueron de Cusiri, alt. 4310 m, 5 Mar 1973, *Cleef 8785* (U); Páramo de la Sarna entre Sogamoso y Vado Hondo, alt. 3330 m., 8 Apr 1973, *Cleef 9522* (COL, U). **Departamento Cundinamarca:** Páramo de

Sumapaz, Chisaca, 100 m SW de la Laguna Larga, alt. 3700 m, 11 Dec 1971, *Cleef 153* (COL, U); Páramo de Cruz Verde, alt. 3200 m, 16 Oct 1961, *Idrobo* & *Hotheway 4174* (COL); Páramo de Sumapaz, vicinity of Laguna Chisaca, pool 50 m S of Laguna Larga, alt. 3620 m, 7 Jul 1986, *Keeley* & *Keeley 11032* (MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, pool 50 m S of Laguna Larga, alt. 3620 m, 7 Jul 1986, *Keeley* & *Keeley 11033* (MU). **Departamento Meta:** Páramo de Sumapaz, Cerro Nevado del Sumapaz, alt. 3725 m, 21 Jan 1973, *Cleef 8185A* (COL).

VENEZUELA. **Estado Mérida:** Sierra de Sto. Domingo, Páramo de Mucubaji, alrededores de la Laguna Grande, alt. 3560–3600 m 19 Nov 1959, *Barclay* & *Juajibioy 9588* (COL, GH).

4a. **Isoëtes karstenii** A. Braun var. **karstenii.** Verh. Bot. Vereins Prov. Brandenberg 3/4:332. 1862. Figs. 20–21.

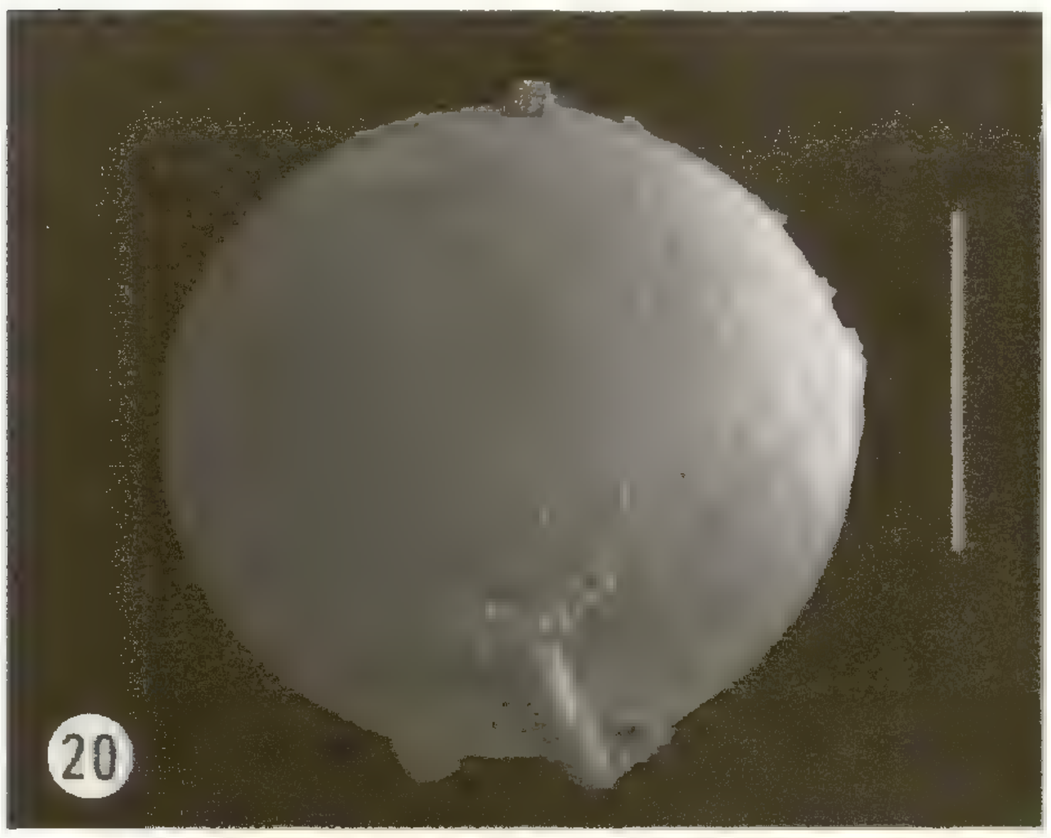
Type: "Venezuela. Im Gebirge von Mérida 8000' hoch in einem See ganz unter Wasser 1857." *Karsten s.n.* (Holotype B!).

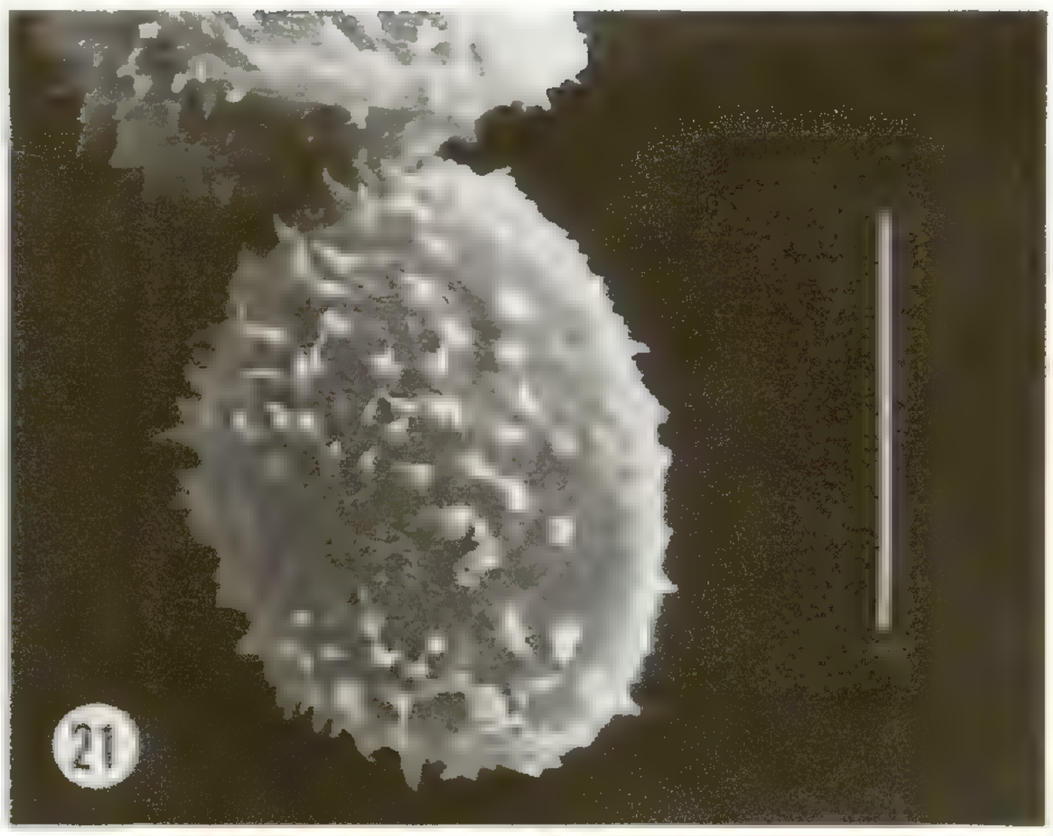
Isoëtes lechleri var. *colombiana* T. C. Palmer, Amer. Fern J. 19: 18. 1929. *I. colombiana* (T. C. Palmer) H. P. Fuchs, Proc. Kon. Ned. Akad. Wetensch. C. 85(2):259. 1982. Type: "Colombia, Dept. Santander: Laguna de Cunta, edge of Páramo de Santurban; alt. 3,880 m, 21 Jan 1927" *Killip & Smith 17964* (Holotype PH; Isotype: NY!), mixed collection.

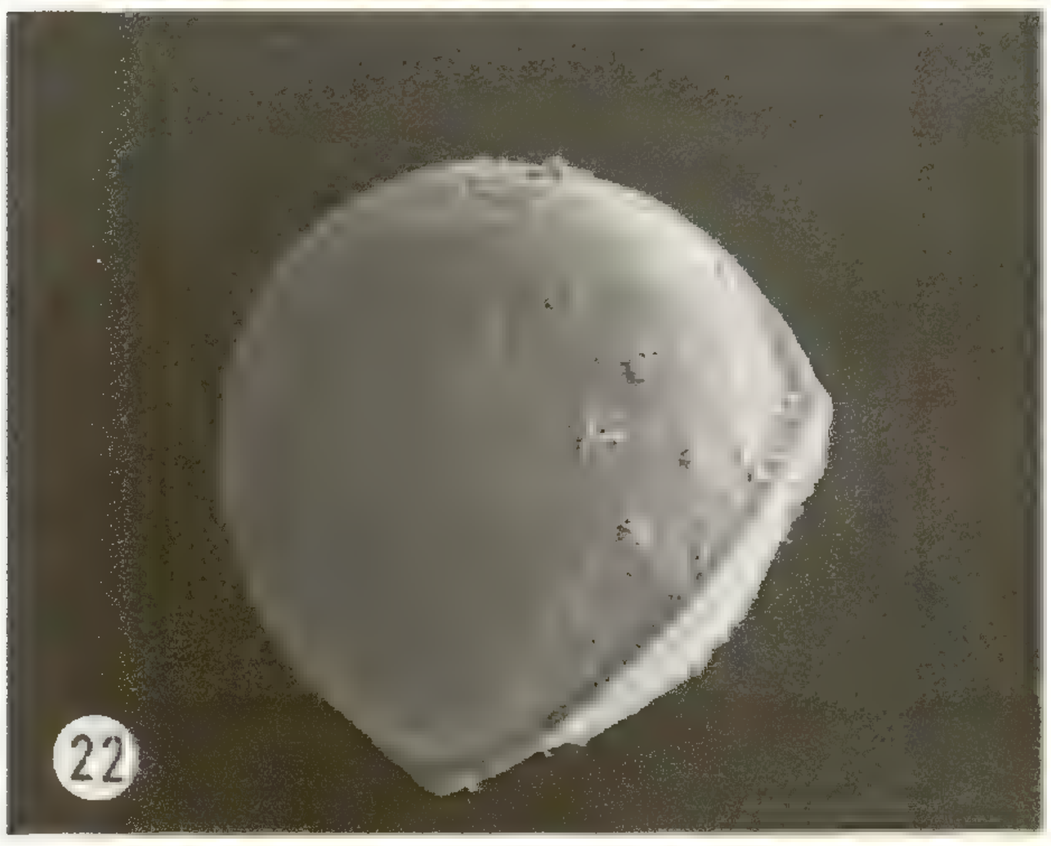
Isoëtes steyermarkii Fuchs-Eckert, nom. nud. Proc. Koninkl. Ned. Acad. Wetensch. C85:257. 1982. Based on *Steyermark 55904* (US!).

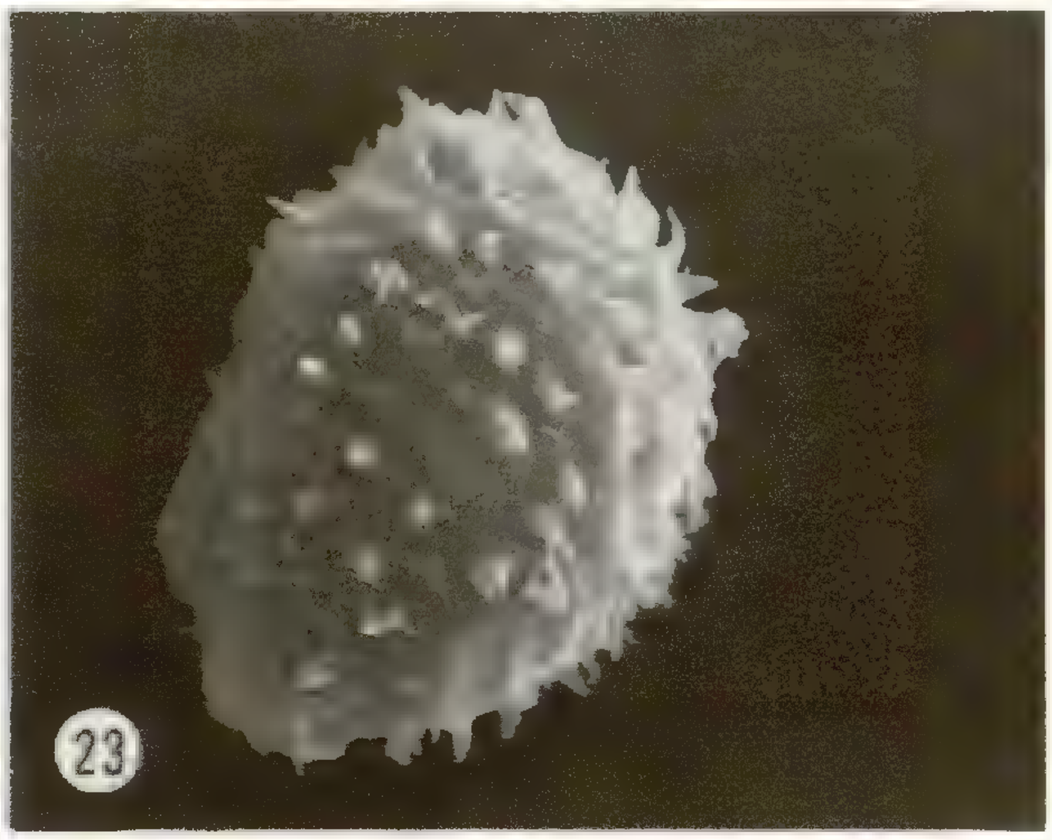
Corm globose to horizontally elongate, two lobed (rarely 3), 2.0–4.0 mm (x = 2.8) high, 2.8–7.8 mm (x = 5.2) wide; dichotomously branching roots arising from the circumbasal fossa(e). Leaves 8–21, flexuous to stiffly erect, to 251 mm long, 5–10 mm wide at base, 0.9 to 2.4 mm wide at mid-length, leaf length/width ratio 35–157 (x = 77); alae chartaceous, light-brown to blackish-brown, extending 14–38% of total leaf length, apices acute to truncate; subula essentially terete in cross-section, olive green, apices blunt, often spatulate, distal 0.5–3.2 mm of apex corneous; peripheral fibrous bundles, stomates, scale leaves and phyllopodia absent. Sporangia widely elliptic to circular, basal, unpigmented, 2.1–5.5 (x = 3.4) mm long, 1.8–3.9 (x = 2.6) mm wide. Velum complete, covering 100% of the sporangial surface (rarely slightly less). Ligule deltate, auriculate, 0.5–1.5 mm high, 0.75–2.0 mm wide. Labium absent or reduced to an extremely small transverse ridge on lower lip of the foveola. Megaspores white, lustrous, 400–507 (x = 467) μm in diameter, laevigate on both proximal and distal faces. Microspores cocoa brown, 25–38 (x = 30) μm in length, 17.7–28.7 (x = 20.8) μm wide, echinate. Chromosome number: $2n$ = 22.

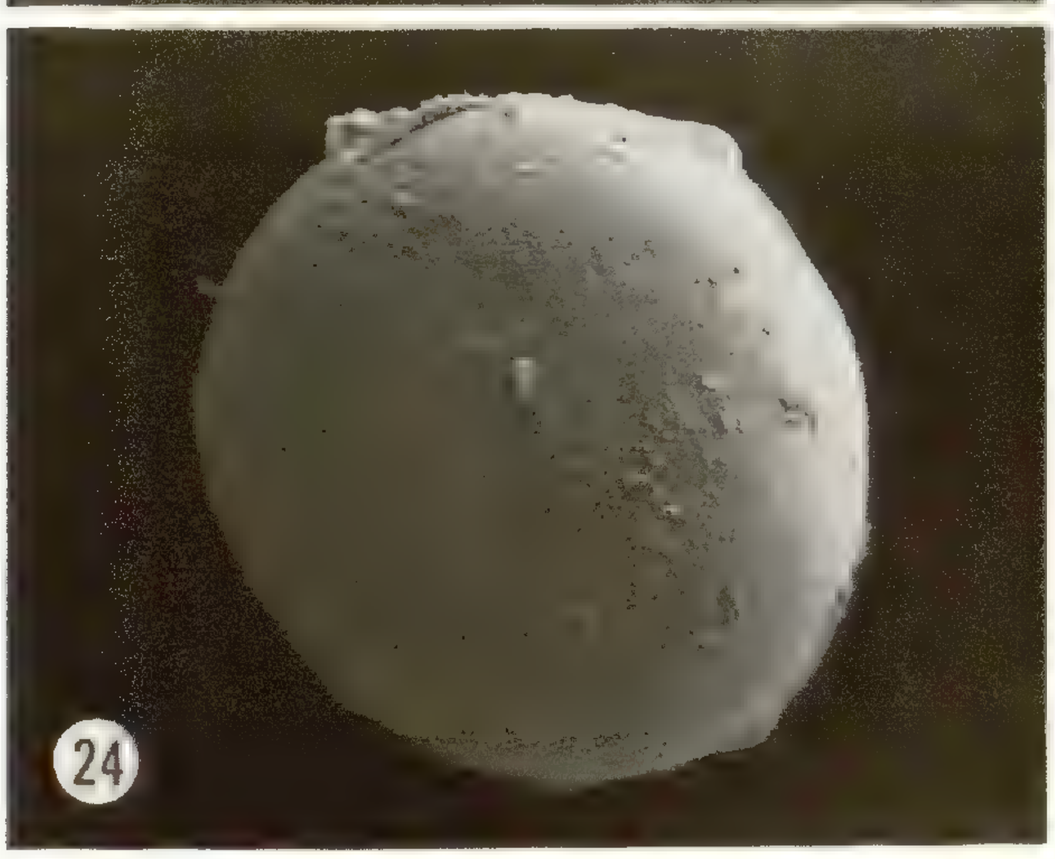
Isoëtes karstenii grows submerged in permanent lakes and ponds (occasionally streams) between 3300–4600 m. It is distributed throughout the eastern cordillera of the northern Andes from Estado Mérida, Venezuela, to Departamentos Arauca, Boyaca, Cundinamarca, Meta, Tolima and Nariño in Colombia (Fig. 8). The collections of this species were made from every month except August and October. With the exception of the single collection made in June,

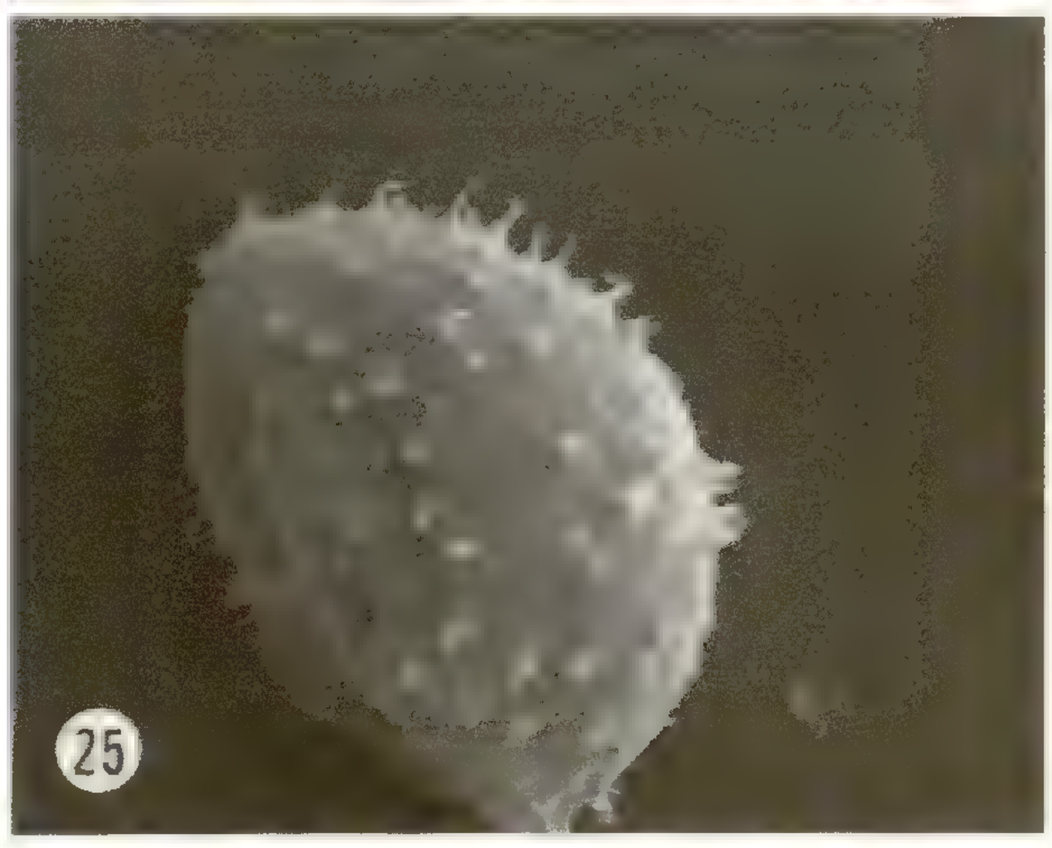












FIGS. 20–25. *Isoëtes karstenii* var. *karstenii*, *I. karstenii* var. *anomala*, and *I. palmeri*. FIGS. 20–21—SEM of spores of *Isoëtes karstenii* var. *karstenii*. FIG. 20. Near equatorial view of megaspore (*Cleef 8599*, BM). Bar = 200 μm. FIG. 21. Equatorial view of microspore (*Oberwinkler & Oberwinkler 13685*, M). Bar = 20 μm. FIGS. 22–23—SEM of spores of *I. karstenii* var. *anomala* (*Rimbach 171*, US). FIG. 22. Distal view of megaspore. Scale as in Fig. 20. FIG. 23. Equatorial view of microspore. Scale as in Fig. 23. FIGS. 24–25—SEM of spores of *I. palmeri* (*Cleef 8647*, BM). FIG. 24. Distal view of megaspore. Scale as in Fig. 20. FIG. 25. Equatorial view of microspore. Scale as in Fig. 21.

specimens from all other months contained both mega- and microspores, suggesting this species is evergreen and fertile year-round. This species is the most widely distributed (or at least most widely collected) member of this group.

Isoëtes colombiana (T. C. Palmer) H. P. Fuchs and *I. steyermarkii* H. P. Fuchs nom. nud. are synonyms of *I. karstenii*. Palmer (1929) originally separated *I. lechleri* var. *colombiana* from *I. lechleri* var. *lechleri* on the basis of the absence of an equatorial ridge on the megaspores, the small size of the microspores, and the corneous leaf apices. All of these characters agree with the current circumscription of *I. karstenii*. In addition, the type collection of *I. lechleri* var. *colombiana* is a mixture of fertile *I. karstenii* and plants of apparent hybrid origin. The hybrids are the specimens that contain ornamented and misshapen megaspores as noted in Palmer's (1929) protologue. *Isoëtes steyermarkii* was invalidly published by Fuchs-Eckert (1982) and no description was given. Examination of the basis for this taxon (*Steyermark 55904*) reveals no characters that differentiate it from *I. karstenii*; in fact this collection is nearly identical to the type of *I. karstenii*.

Finally, the type specimen of *I. karstenii* as cited by Braun (1862) is "In einem ungefahr 8000' hoch See auf dem Páramo im Gebirge von Mérida (Venezuela) ganz unter Wasser im Jahre 1853 von Prof. H. Karsten entdeckt,. . ." The specimen at Berlin which was annotated as the holotype by H. P. Fuchs bears the label "*Isoëtes karstenii* A. Braun. Venezuela. Im Gebirge von Mérida 8000' hoch in einem See ganz unter Wasser. C. Dr. Karsten etc. 1857." Although the locality information on this label closely matches that of Braun's (1862) description, there is a discrepancy regarding the date of collection: 1853 in the description and 1857 on the specimen label. Stafleu and Cowan (1979) indicate that Karsten was in South America from 1848 to 1856, but returned to Berlin in 1856 where he remained until 1868. Thus, he could not have collected this specimen in 1857. A potential explanation for this discrepancy could be that 1853 was the date of collection, while 1857 was the date the material was accessioned into the Berlin herbarium.

Additional specimens examined:

COLOMBIA. **Departamento Arauca:** Cordillera Oriental, extremo sur de la Sierra Nevada del Cocuy, Laguna el Amarillal, alt. 4065 m, 23 Mar 1977, *Cleef* & *van der Hammen 10383* (U); Sierra Nevada del Cocuy, Laguna de la Plaza, alt. 4550 m, 20–30 Jan 1959, *van der Hammen* & *Gonzalez 1374* (COL); Sierra Nevada del Cocuy, Laguna la Plaza, alt. 4150 m, Mar 1977, *van der Hammen 4753* (COL, U). **Departamento Boyacá:** Sierra Nevada del Cocuy, Alto Valle Lagunillas, Laguna Cuadrada, alt. 4060 m, 26 Sept. 1972, *Cleef* & *Florschütz 5590* (COL, U); Sierra Nevada del Cocuy, Páramo Concavo, 3 km al N del morro Pulpito del Diable, alt. 4410 m, 27 Feb 1973, *Cleef 8599* (U); Sierra Nevada del Cocuy, Alto Valle Lagunillas, medio km al WNW de la Laguna Pintada, alt. 4000 m, 4 Mar 1973, *Cleef 8782* (COL, U); Municipio de El Cocuy, valle del rio Lagunillas, Sierra Nevada del Cocuy, Laguna La Pintada, alt. 3800 m, 23 May 1993, *Small, Gonzalez* & *Ruiz 141–143* (COL, MU); Municipio de El Cocuy, valle del rio Lagunillas, Sierra Nevada del Cocuy, Laguna La Cuadrada, alt. 3900 m, 23 May 1993, *Small, Gonzalez* & *Ruiz 144* (COL, MU). **Departamento Cundinamarca:** Páramo de Sumapaz, Chisaca, Laguna Negra, alt. 3750 m, 11 Dec 1971, *Cleef 174* (COL, U); Km 31, south of Usme, Laguna Chisaca, alt. 3620 m, 3 Jan 1985, *Keeley* & *Keeley 7908* (COL, MU); Km 31, south of Usme, Laguna Chisaca, alt. 3620 m, 3 Jan 1985, *Keeley* & *Keeley 7909* (COL, MU); Small laguna adjacent to north side of road at km 31 between Laguna Chisaca and Laguna Larga, alt. 3650 m, 10 Dec 1985, *Keeley* & *Keeley 10076* (MO, MU); Unnamed laguna on

opposite side of road from largest laguna at km 30.5, Páramo de Sumapaz, vicinity of Laguna Chisaca, alt. 3650 m, 10 Dec 1985, *Keeley* & *Keeley 10082* (MO, MU); Páramo de Sumapaz, laguna at km 30.5 opposite side of road from Laguna Chisaca, alt. 3700 m, 15 Dec 1985, *Keeley* & *Keeley 10093* (MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, laguna adjacent to road at km 31, alt. 3620 m, 7 Jul 1986, *Keeley* & *Keeley 11037* (MU); Páramo, alt. 3700 m, Apr 1963, *Saravia 2533* (COL). **Departamento Meta:** Páramo de Sumapaz, Laguna La Guitarra, alt. 3460 m, 21 Jan 1972, *Cleef 832* (COL, U); Páramo de Sumapaz, Laguna La Primavera, alt. 3525 m, 25 Jan 1972, *Cleef 1017* (COL, U); Páramo de Sumapaz, Cerro Nevado del Sumapaz, alt. 4050 m, 30 Jan 1972, *Cleef 1351* (COL, U); Páramo de Sumapaz, Cerro Nevado del Sumapaz, alt. 4090 m, 13 Jan 1973, *Cleef 7766* (COL, U); Páramo de Sumapaz, Laguna Los Sitiales, alt. 3620 m, 22 Jan 1973, *Cleef 8248* (COL, U); Macizo de Sumapaz, Boqueron del Palacio, norte de Cerro del Nevado, alt. 3900 m, 5 Jul 1981, *Diaz, et al. 2558* (COL); Macizo de Sumapaz, Laguna La Guitarra, alt. 3370 m, 2 Jun 1981, *Diaz* & *Rangel 2390* (COL). **Departamento Nariño:** Cumbal, Laguna La Bolsa, alt. 3400 m, 21 Jan 1973, *Hagemann* & *Leist 1968* (COL); Laguna Negra en vecindades de Pasto, alt. 3520 m, 17 Jul 1973, *Leist* & *Möhle 2212* (COL).

VENEZUELA. **Estado Mérida:** Laguna Grande de Mucubaji, alt. 3535 m, 23 Dec 1984, *Keeley* & *Keeley 7861* (MU); Laguna Grande de Mucubaji, alt. 3535 m, 23 Dec 1984, *Keeley* & *Keeley 7862* (MU); Laguna Negra, alt. 3430 m, 23 Dec 1984, *Keeley* & *Keeley 7865* (MU); Laguna de los Patos, alt. 3670 m, 23 Dec 1984, *Keeley* & *Keeley 7868* (MU); Laguna Negra, alt. 3400 m, 22 Nov. 1968, *Oberwinkler* & *Oberwinkler 13685* (M); Laguna St. Barbara, alt. 3700 m, 11 Jan 1969, *Oberwinkler* & *Oberwinkler 14272* (M); Laguna St. Barbara, alt. 3700 m, 11 Jan 1969, *Oberwinkler* & *Oberwinkler 14274* (M); Small pond ca. 8 km N of Pico de Aguila on road to Pinango, alt. 3500 m, 15 Mar 1992, *Small & Hickey 4* (MU); Small pond ca. 15 km N of Pico de Aguila on road to Pinango, alt. 3500 m, 15 Mar 1992, *Small & Hickey 5* (MU); Small pond ca. 15 km N of Pico de Aguila on road to Pinango, alt. 3500 m, 15 Mar 1992, *Small & Hickey 6* (MU); Laguna Negra, alt. 3500 m, 17 Mar 1992, *Small & Hickey 7* (MU); Laguna Mucubaji, alt. 3500 m, 17 Mar 1992, *Small & Hickey 8* (MU); Near upper limits of páramo, around small lake between Chachapo and Los Apartaderos, near El Aguila, 3930 m, 15 Apr 1944, *Steyermark 55904* (F, US, VEN); Laguna Grande de Mucubaji, alt. 3500 m 19 Sep 1961, *Tryon* & *Tryon 5839* (GH).

4b. **Isoëtes karstenii** var. **anomala** (T. C. Palmer) R.L. Small and Hickey, **comb. nov.** Figs. 22–23.

Isoëtes lechleri var. *anomala* T. C. Palmer, Amer. Fern J. 22: 130–131. 1932. *Isoëtes rimbachiana* H. P. Fuchs Proc. Kon. Ned. Akad. Wetensch. C. 85(2): 259. 1982. Type: "Ecuador. Mt. Chimborazo. Submerged in water of shallow pond. Rooting in mud. 4200 m." *Rimbach 171* (Holotype US!)

Isoëtes karstenii var. *anomala* differs from *I. karstenii* var. *karstenii* only in the character of velum coverage: 25–75% in var. *anomala*, >90% in var. *karstenii*. In Palmer's (1932) description of this taxon (as *I. lechleri* var. *anomala*) he distinguishes it from other "*I. lechleri*" (of which he considered *I. karstenii* to be a synonym) based primarily on the presence of a two-layered velum which incompletely covered the sporangium. Examination of the holotype, however, revealed no indication of a two-layered velum on the sporophylls we observed. The presence of a "multilayered velum" has been reported elsewhere (e.g., *Isoëtes prototypus*, Britton and Goltz, 1991). Initial investigations indicate that the apparent multilayered velum is actually due to disintegration of the mesophyll of the velum and separation of the upper and lower epidermal layers resulting in what appears as a double velum (Hickey, unpublished data). Regardless, this taxon appears unique in the extent of velum coverage.

It is otherwise nearly identical to the type of *I. karstenii,* as noted by Palmer (1932). This taxon is known only from the type locality (Mt. Chimborazo, Ecuador) although Rodriguez (1955) cites one specimen from Laguna Negra, Mérida, Venezuela as *I. lechleri* var. *anomala.*

5. **Isoëtes palmeri** H. P. Fuchs. Proc. Kon. Ned. Akad. Wetensch. C. 84 (2): 168–173. 1981. Figs. 24–25.

Type: "Venezuela; Laguna Grande de Apartaderos, Mérida, floating on water, 21 January 1929" *Pittier 13242* (Holotype US!; Isotypes G!, M!, MO!, VEN!).

Isoëtes cleefii H. P. Fuchs Proc. Kon. Ned. Akad. Wetensch. C. 84 (2):177–181. 1981. Type: "Colombia, Cundinamarca: Páramo de Sumapaz, páramo y bosque alto-andino cerca de Lagunitas al S. de San Juan. Laguna Gobernador: orilla W." *Cleef 8308* (Holotype COL!; Isotype COL!).

Corm globose to horizontally elongate, two lobed, 1.3–6.0 mm (x = 2.9) high, 3.0–8.5 mm (x = 5.0) wide; dichotomously branching roots arising from the circumbasal fossa. Leaves 5–25, flexuous, to 350 mm long, 5–8 mm wide at base, 0.5 to 1.5 mm wide at mid-length, leaf length/width ratio 118–350 (x = 241); alae chartaceous, dark brown to blackened distally, pale proximally where leaf was imbedded in substrate, extending 10–27% of total leaf length, apices acute to truncate; subula essentially terete in cross-section, pale green, apices blunt, often spatulate, distal 1.0–2.5 mm of apex sclerified and blackened; peripheral fibrous bundles, stomates, scale leaves and phyllopodia absent. Sporangia elliptic to obovate, basal, unpigmented, 2.5–5.9 (x = 3.8) mm long, 1.9–4.0 (x = 2.8) mm wide. Velum complete, covering 100% of the sporangial surface (rarely slightly less). Ligule deltate, auriculate, to 1 mm high, to 1 mm wide. Labium absent or reduced to an extremely small transverse ridge on lower lip of the foveola. Megaspores white, lustrous, 380–550 (x = 460) μm in diameter, laevigate on both proximal and distal faces. Microspores cocoa brown, 26–36 (x = 30.4) μm in length, 17.9–26.4 (x = 21.3) μm wide, echinate. Chromosome number: $2n = 44$.

Isoëtes palmeri grows submerged in permanent pools, ponds, and lakes between 3340–4435 m. It is distributed in the eastern cordillera of the northern Andes from Estado Mérida, Venezuela, through the Departamentos Arauca, Boyaca, Cundinamarca and Meta of Colombia (Fig. 8).

The presence of both microspores and megaspores in all months for which collections are available (nine out of twelve) suggests that this species is evergreen and fertile year-round.

Isoëtes palmeri's affiliation with the *I. karstenii* complex is apparent by its laevigate megaspores, acute to truncate alae apices, and corneous leaf tips. For the most part, *I. palmeri* and *I. karstenii* are easily differentiated morphologically; *I. palmeri* has long, slender leaves which appear flexuous, and *I. karstenii* has shorter, wider leaves which usually appear rigid and stiff. Occasional collections appear intermediate between these two extremes. These col-

lections often represent juvenile individuals of either species, but may also include hybrids.

Isoëtes cleefii is a synonym of *I. palmeri*. Although the holotype and one of the paratypes of *I. cleefii* have leaves that are slightly longer and wider than typical *I. palmeri*, these individuals are merely extreme variants within a wide range of intraspecific variation. Both *I. palmeri* and *I. cleefii* were published by Fuchs-Eckert (1981a,b) at the same time, thus neither name has priority. We select the name *I. palmeri* to represent this species because the type collection is more representative of the species.

Additional speciemens examined:

COLOMBIA. **Departamento Arauca:** Sierra Nevada del Cocuy, Cabeceras de la Quebrada El Playon, alt. 4245 m, 11 Mar 1973, *Cleef 9088* (COL, U). **Departamento Boyacá:** Páramos al NW de Belen, ca. 600 m NE de la Laguna el Alcohol, alt. 3850 m, 29 Feb 1972, *Cleef 2059* (COL,U); Páramo de la Rusia, NW-N de Duitama, alt. 3570 m, 13, Dec 1972, *Cleef 7144* (COL, U); Sierra Nevada del Cocuy, Páramo Concavo, alt. 4435 m, 28 Feb 1973, *Cleef 8647* (COL, U); Páramo de la Rusia, NW-N de Duitama, Laguna Negra, alt. 3745 m, 14 Dec 1972, *Cleef 7221* (COL, U); Sierra Nevada del Cocuy, Páramo Concavo, 2.5 km al N de morro Pulpito del Diablo, alt. 4435 m, 28 Feb 1973, *Cleef 8648* (COL, U); Páramo entre Peña Arnical y Alto de Mogotes; lagunita 0.5 km al SE de la Laguna Grande, alt. 3340 m, 1 Apr 1973, *Cleef 9273* (COL, U); Páramo entre Pena de Arnical y Alto de Mogotes, cerca de la Laguna Grande, alt. 3350 m, 10 Apr 1973, *Cleef 9551* (COL, U); Páramos al NW de Belen, vereda San Jose de la Montana, alt. 3725 m, 3 May 1973, *Cleef 9699* (U); Páramo de Pisva, carretera Socha-La Punta km 77, Laguna Colorada, alt. 3425 m, 22 May 1973, *Cleef 9898* (COL, U); Largest laguna, 17 km NW of Belen on road to San Jose de Montana, alt. 3700 m, 1 Jan 1985, *Keeley* & *Keeley 7887* (COL, MU). **Departamento Cundinamarca:** Páramo entre Cogua y San Cayetano, Laguna Verde, 3600 m, 30 Nov 1971, *Cleef 80* (COL,U); Páramo de Sumapaz, hoya la Laguna Larga, alt. 3750 m, 14 Dec 1971, *Cleef* & *Jamarillo 262* (COL, U); Páramo de Palacio, Lagunas Buitrago, alt. 3600 m, 16 Dec 1971, *Cleef 296* (COL, U); Páramo de Sumapaz, Andabobos, alt. 3800 m, 9 Feb 1972, *Cleef 1553* (COL, U); Páramo de Palacio, Lagunas de Buitrago, alt. 3580 m, 25 May 1972, *Cleef 4112* (COL, U); Páramo de Sumapaz, lagunitas al S de San Juan, Laguna Gobernador, alt. 3815 m, 26 Jan 1973, *Cleef 8308* (COL); Páramo de Chingaza, cerca de la "Paila", 10–20 Jan 1965, *Huertas* & *Camargo 6025* (COL); Cordillera Oriental, Macizo de Sumapaz, Laguna de Chisaca, alt. 3680 m, 14 Apr 1958, *Idrobo 2741* (COL); Laguna Seca, N of Zipaquire, alt. 3700 m, 30 Dec 1984, *Keeley* & *Keeley 7876* (MU); Laguna Seca, N of Zipaquire, alt. 3700 m, 30 Dec 1984, *Keeley* & *Keeley 7878* (COL, MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, Laguna Larga, alt. 3650 m, 10 Dec 1985, *Keeley* & *Keeley 10072* (MO, MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, Laguna Larga, alt. 3650 m, 10 Dec 1985, *Keeley* & *Keeley 10073* (MO, MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, small pools S of Laguna Larga, alt. 3650 m, 10 Dec 1985, *Keeley* & *Keeley 10077* (MO, MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, small pools ca. 100 m above SE end of Laguna Chisaca, alt. 3700 m, 13 Dec 1985, *Keeley* & *Keeley 10088* (MO, MU); Páramo de Sumapaz, Laguna Chisaca, alt. 3700 m, 13 Dec 1985, *Keeley* & *Keeley 10090* (MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, small pool ca. 200 m above SE end of laguna on NE side of road at km 30.5, alt. 3700 m, 13 Dec 1985, *Keeley* & *Keeley 10091* (MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, laguna ca 100 m NE of km 31 lagunita, alt. 3700 m, 13 Dec 1985, *Keeley* & *Keeley 10092* (MO, MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, Laguna Larga, alt. 3620 m, 7 Jul 1986, *Keeley* & *Keeley 11029* (MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, shallow pool on ridge SE of first lake, alt. 3620 m, 7 Jul 1986, *Keeley* & *Keeley 11039* (MU); Carretera Cogua-San Cayetano, Páramo de Laguna Seca, alt. 3660 m, 3 Aug 1970, *Piedrahita 276* (COL). **Departamento Meta:** Páramo de Sumapaz, Laguna la Primavera, alt. 3510 m, 9 Jan 1973, *Cleef 7556* (COL, U); Páramo de Sumapaz, Lagunas el Sorbedero y El Nevado, alt. 3635 m, 16 Jan 1973, *Cleef 7910* (COL, U); Páramo de Sumapaz, Laguna la Guitarra, alt. 3425 m, 23 Jan 1973, *Cleef 8267* (COL).

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American Fern Journal 91(2):70–72 (2001)

Shorter Note

Additions and Corrections to the Pteridophyte Flora of Northeastern Argentina.—Two new records as well as material to confirm two non-vouchered records were collected by colleagues at the Instituto Darwinion and Parques Nacionales during botanic expeditions to Misiones in northeastern Argentina. Misiones is a strip of land between the Uruguay and Paraná rivers, with longitudinal mountains reaching approximately 800 m in elevation. This region is under exploration in order to study and document the floristic composition of the southernmost subtropical forests in South America. These records are elements of the *Araucaria angustifolia* forests and rocky "campos", two primary communities that should be maintained and preserved within national and state reserves.

Megalastrum crenulans (Fée) A. R. Smith & R. C. Moran, Amer. Fern J. 77: 127. 1988.

Aspidium crenulans Fée, Cr. vasc. Brés. 1: 139, t. 47, f. 1, 1869.

Dryopteris crenulans (Fée) C. Chr.

Descriptions and Iconographies.—Fée, op. cit.; Sehnem, Aspidiáceas, 186, f. 45, in R. Reitz (ed.) *Flora Ilustrada Catarinense* Parte I (ASPI) Itajaí, SC, Brasil, 1979.

Distribution and Ecology.—This fern grows in southern Brasil, Paraguay and has been cited also as occuring in the Venezuelan Guyana (Smith, Pteridophytes pp. 1–334, in A. Steyermark, P. E. Berry & B. K. Holst (Gen. Eds.) *Flora of the Venezuelan Guayana* 2, Missouri Botanical Garden, 1995). Here it is recorded for the first time for Argentina, elevating the number of species of *Megalastrum* in this country to five (Ponce, Monogr. Syst. Bot. Missouri Bot. Gard. 60: 1–79. 1996). It inhabits subtropical Araucaria forests.

This species is characterized by the occurrence of glandular trichomes mixed with simple trichomes, both of which are short and dense on the abaxial face, bulliform scales on the costule, and large, brown and glandular indusia. The latter character is not common in *Megalastrum*. The forms *crenulans* and *glandulosa* (Rosent.) C. Chr. were described by Christensen (Kongel. Danske Vidensk. Selsk. Skr. Naturvidensk. Afd. 8(6): 3–132. 1920); material from Misiones is referable to the latter. A revision of the genus is necessary to clarify species boundaries.

Specimen Studied.—**ARGENTINA. Misiones,** Dpto. San Pedro, Parque Provincial Cruce Caballero, 690 m, 26° 31′ S, 53° 59′ W, 15-IV-1996, *F. O. Zuloaga, O. Morrone & M. Múlgura 5551* (SI).

Elaphoglossum pachydermum (Fée) T. Moore, Index Fil. 12. 1857.

Acrostichum pachydermum Fée, 2ème. Mém. Foug. 47. 1845.

DESCRIPTIONS AND ICONOGRAPHY.—Sehnem, Aspidiáceas, 24, Fig. 5.2, in R. Reitz (ed.) *Flora Ilustrada Catarinense* Parte I (ASPI) Itajaí, SC, Brasil, 1979.

DISTRIBUTION AND ECOLOGY.—This species grows in Brasil, Paraguay and is here reported for Argentina. It is rupiculous, occurring on rocky promontories and steep slopes in montane areas.

This species has erect to short creeping rhizomes and fimbriate scales from the base to the middle of the petiole. The sterile fronds are longer than the fertile fronds, the lamina is linear-oblong, attenuate-decurrently based, coriaceous in texture, and with minute, reddish to brown, stellate trichomes on the abaxial surface.

Elaphoglossum pachydermum is in the *E. latifolium* (Sw.) J. Sm. complex, and was thought to be confined to the Antilles (Mickel, pers. comm.). Previously it was recorded for Argentina by Hassler (Trab. Inst. Bot. Farmacol. 45: 1–102. 1928) as *E. latifolium*, but the voucher specimem (*Parodi 84*) could not be located for verification.

With this report, *E. pachydermum* is documented for the first time for Argentina. The related species, *E. macrophyllum* (Mett. *ex* Kuhn) H. Christ and *E. macahense* (Fée) Rosenst., are known from Brasil.

SPECIMEN STUDIED.—**ARGENTINA. Misiones.** Dpto. San Ignacio, Parque Provincial Teyú Cuaré. Peñón del Teyú Cuaré, rupícola en zonas protegidas, 28/09/1998, *Biganzoli, F. & D. Giraldo-Cañas 394* (SI).

Cheiroglossa palmata (L.) C. Presl, Suppl. Tent. Pterid. 57. "1845" 1846.
Ophioglossum palmatum L. Sp. Pl. 1063. 1753.

DESCRIPTION AND ICONOGRAPHY.—Tryon & Stolze (Fieldiana, Bot. n.s. 20: 9, f. 2. 1989).

DISTRIBUTION AND ECOLOGY.—This fern grows in southern Florida (USA), the Antilles, southern Mexico to Brazil, and northeastern Argentina. It is also disjunct in Madagascar and southeastern Asia. It is epiphytic and suberect to pendant on trunks in subtropical primary and marginal forests.

Wagner's treatment of the Ophioglossaceae (Ophioglossaceae, pp. 44–48, in G. Davise et al. (Eds.) *Flora Mesoamericana.* Vol. 1, Univ. Nac. Autónoma de México, 1995) is accepted here. Wagner considers *Cheiroglossa* a genus justifiably separated from *Ophioglossum.* This species was cited by Molfino (Physis, Buenos Aires, 8: 259–260. 1925) and Lichtenstein (Darwiniana 6: 380–441. 1944) as being in Misiones, but without documenting herbarium specimens.

SPECIMEN STUDIED.—**ARGENTINA. Misiones.** *Dpto. Gral. Manuel Belgrano*: Deseado, Reserva Privada Caá-porá, epífita sobre "Sotacaballo", invierno/1996, *Chaves & Chebez s.n.* (SI).

Dicranopteris flexuosa (Schrad.) Underw., Bull. Torrey Bot. Club 34: 254. 1907.
Mertensia flexuosa Schrad., Gött. gel. Anz. 1824: 863. 1824.

DESCRIPTIONS AND ICONOGRAPHS.—Fée, Crypt. vasc. bras. 1: 199, t. 72, f. 1 (sub *Mertensia scalpturata* Fée); 200, t. 73, f. 2 (sub *M. spissa* Fée) 1869. Sehnem,

Gleiqueniáceas, in R. Reitz (ed.) *Flora Ilustrada Catarinense* Parte I (GLEI): 25, f. 9. 1970.

DISTRIBUTION AND ECOLOGY.—Southern Alabama (USA), the Antilles, southern Mexico, Central America, Colombia and Guyanas to Bolivia, southern Brazil, Paraguay, and Argentina. Terrestrial and rupiculous at shady and moist sites in rocky "campos" and marginal forests.

Dicranopteris flexuosa is the correct name for the neotropical counterpart of the paleotropical *D. linearis* (Burm.) Underw. Roth (Faculty of Natural Sciences, University of Götreborg, Sweeden, 1986), in her thesis on the neotropical Gleicheniaceae, suggested that *D. flexuosa* may represent a subspecies of *D. linearis,* but that combination has never been made.

SPECIMEN STUDIED.—**ARGENTINA. Misiones,** Dpto. San Ignacio, Parque Provincial Teyú Cuaré. Peñón del Teyú Cuaré, rupícola, ocasional en zona protegida y húmeda, localmente abundante, 28/09/1998, *Biganzoli, F. & Giraldo-Cañas 397* (SI).

Thanks to colleagues and institutions of the Inventario Florístico de la Provincia de Misiones project (PMT-PICT 01511) directed by F. Zuloaga and supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) Argentina.—MÓNICA PONCE, Instituto de Botánica Darwinion, Labardén 200, B1642HYD San Isidro, Argentina.

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American Fern Journal 91(3):73 (2001)

The Evolution and Diversification of the Lypopods

The lycopods, a diverse and ancient group of pteridophytes, form a major lineage of vascular plants. This lineage includes three orders, Lycopodiales, Selaginellales, and Isoetales. Each order contains a single extant family, but the group has a long and diverse fossil record beginning in the Devonian or possibly late Silurian. This fossil record is replete with many curious forms that have long captured the interest and imagination of those interested in plant evolution. Although lycopods have attracted the attention of paleobotanists and morphologists for many years, it is only within the last few years that systematists have initiated studies to critically evaluate taxa and their modes of speciation. While there is agreement that the lycopods form a clade distinct from all other vascular plants, there is some argument in the interpretation of the diversity and evolution within the lycopod lineage. This is an exciting time for systematic biologists. We are beginning to uncover, discover, and explore the modes and mechanisms of divergent and reticulate evolution in the lycopods, revealing their incredibly long history and amazing diversity.

The following five papers are facsimiles of oral presentations given on 4 August 1999, in a symposium entitled "The Evolution and Diversification of the Lycopods." This symposium, a part of the XVI International Botanical Congress held in St. Louis, Missouri, brought leading researchers together to present their current findings relevant to the phylogeny of the lycopods. Niklas Wikström, Léa Grauvogel-Stamm, and Carl Taylor organized the symposium.

The authors express their gratitude to Brent D. Mishler, the Green Plant Phylogeny Research Coordination Group, the U.S. Department of Agriculture, the National Science Foundation, the Department of Energy, and the organizers of the XVI International Congress for their assistance and support. The authors are also grateful to the American Fern Society, Inc. for sponsoring the publication of their papers.—*W. Carl Taylor,* Department of Botany, Milwaukee Public Museum, Milwaukee, WI 53233

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American Fern Journal 91(3):74–98 (2001)

Early Lycophyte Evolution

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ABSTRACT.—Lycophytes, comprising the groups historically known as the lycopsids and zosterophylls, have the longest history of any group of vascular land plants. The early evolution of the group is reviewed concentrating on the Late Silurian and Devonian record of Lycopsida and Zosterophyllopsida. Distinct root-like and shoot-like axes in zosterophyllopsids and lycopsids are first recorded in the Early Devonian and can be compared to the later development of pseudobipolar growth of tree-shaped lycopsids with upward-growing trunks and branch systems and downward-growing rooting systems. The development of stigmarian rootlets postdates the evolution of pseudobipolar growth, first recorded in the Middle Devonian, according to current evidence. Other important events in early lycophyte evolution during the Devonian include changes in leaf morphology, modification of sporophylls, and the eventual appearance of the lycopsid strobili.

WHAT IS A LYCOPHYTE AND WHEN DID LYCOPHYTES APPEAR?

The lycophytes (= club mosses and related plants) are regarded as a distinct lineage of vascular plants with a long evolutionary history. Considerable diversity is evident for much of their existence, although only four (or 7+ if one accepts either Øllgard's (1987) or Wagner and Beitel's (1992) splitting of *Lycopodium* into several genera) herbaceous genera are recognized today (*Lycopodium* (and segregates), *Phylloglossum, Selaginella,* and *Isoetes*). Club mosses have long been defined as plants with microphyllous leaves, axillary or adaxially borne reniform sporangia with dehiscence along their distal margin, and mostly exarch xylem maturation; such plants are now included in the class Lycopsida ("lycopsids") by Kenrick and Crane (1997). Extinct taxa attributed to Zosterophyllopsida (and possibly including barinophytes) are considered closely related to lycopsids, either as ancestral or sister taxa (Gensel, 1992; Hueber, 1992; Bateman, 1992; Kenrick and Crane, 1997), being similar in sporangium shape and xylem attributes but differing from lycopsids in lacking leaves and leaf-borne sporangia. Additionally they have vascularized sporangial stalks (Fig. 1).

REDEFINITION OF "LYCOPHYTES" AND "LYCOPSIDS".—Several workers emphasize the monophyly of vascular plants (Mishler *et al.*, 1994; Stewart and Rothwell, 1993; Kenrick and Crane, 1997). Many types of evidence, molecular and morphological, demonstrate that lycophytes *sensu lato* form a distinct lineage of vascular plants from early in their evolutionary history (Raubeson and Jansen, 1992; Kranz and Huss, 1996; Kenrick and Crane, 1997). In Kenrick and Crane's recent, extensive morphological phylogenetic analysis of living and extinct plants, monophyly is supported, and zosterophylls and lycopsids are interpreted as two classes in a larger group, subdivision Lycophytina (Fig. 1). This

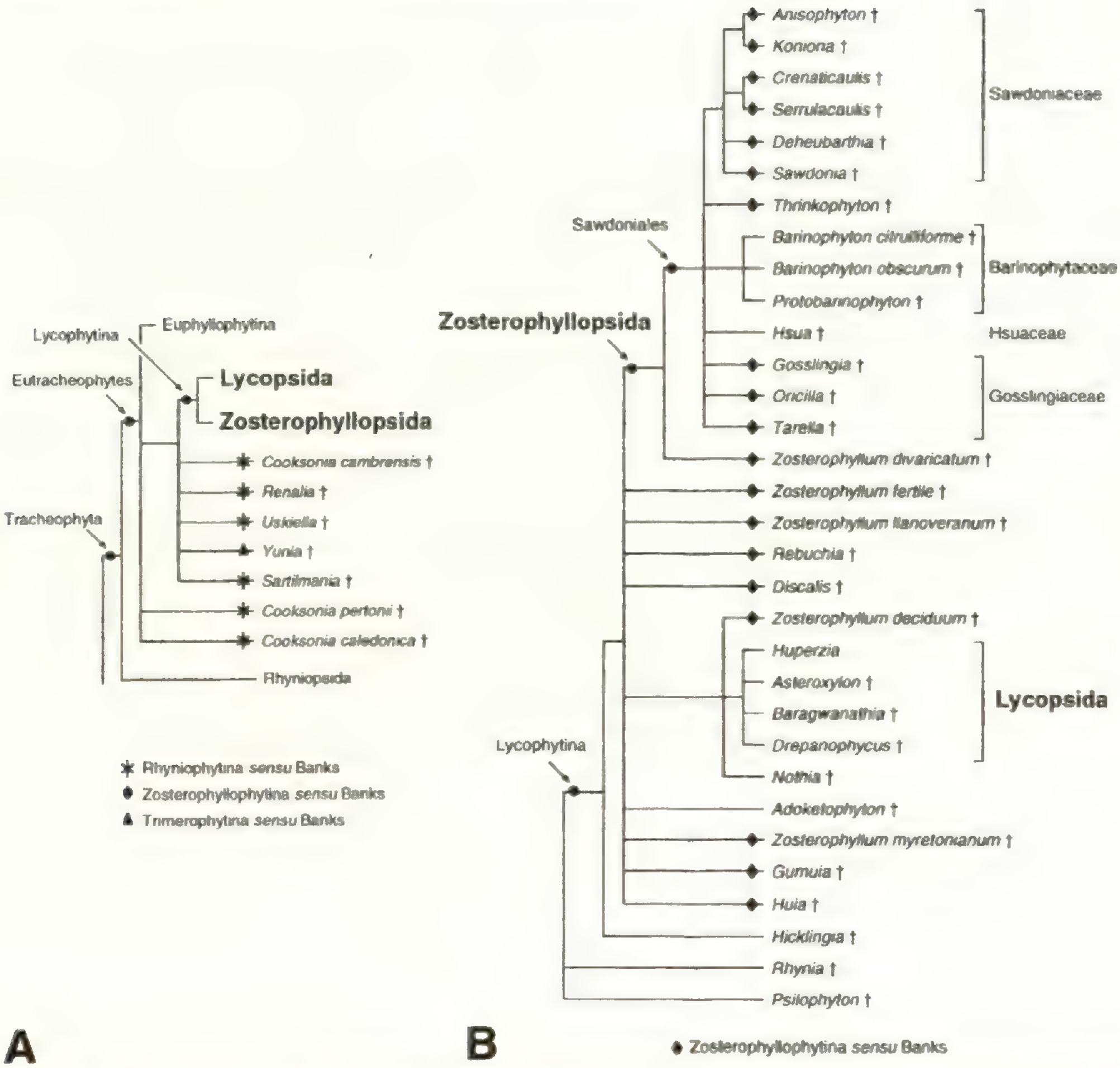

FIG. 1. Strict consensus trees showing relationships among several polysporangiophyte taxa and among lycophytes according to analyses conducted by Kenrick and Crane (1997). A) Adapted from Kenrick and Crane, fig. 4.31. B) From Kenrick and Crane, fig. 5.25, with permission. Zosterophyllopsida and Lycopsida are highlighted. See text for discussion. Not all taxa discussed in the text are included, partly because of differences in preservation and therefore absence of characters for some taxa.

larger group is defined (p.237–238) by the following synapomorphies: "more or less reniform sporangia, marked sporangium dorsiventrality, isovalvate dehiscence along distal sporangium margin, conspicuous cellular thickening along sporangial dehiscence line, sporangia on short, laterally inserted stalks, exarch xylem differentiation." Within the Lycophytina, the strict consensus tree of their analysis (p. 172) shows a polytomy (Fig. 1B) from which emerge the Zosterophyllopsida, a clade largely consisting of lycopsids, and a large number of individual taxa. Zosterophyllopsida *sensu* Kenrick and Crane (Fig. 1B) are defined by only two synapomorphies, namely circinate growth and a unique, two-rowed arrangement of sporangia. Among the single unresolved taxa excluded from Zosterophyllopsida *sensu stricto* are several plant genera (*Rebuchia, Discalis*) originally placed among the zosterophylls, several species

of *Zosterophyllum* including the type species (*Z. myretonianum* Penhallow), and some recently discovered Chinese forms. Thus Kenrick and Crane regard plants traditionally considered zosterophylls as paraphyletic. The incomplete data among the taxa analyzed for all characters employed, and lack of resolution at lower levels in the cladogram, indicates further investigation of characters and character homologies is needed. Thus these conclusions should be regarded with caution until additional data are available.

Kenrick and Crane (1997) define the second clade, the Lycopsida (Fig. 1B), by the synapomorphies of microphylls, stellate xylem strand, vascularized leaves, sporophylls (close association of sporangium and leaf), and loss of sporangial vasculature. Lycopsida includes a clade composed of three possibly transitional genera, *Asteroxylon, Drepanophycus,* and *Baragwanathia,* and a second larger clade of plants traditionally considered as lycopsids. The first clade is composed of taxa that do not possess all of the characters definitive of lycopsids—*Asteroxylon* and *Drepanophycus* have cauline sporangia with a vascularized stalk, and *Asteroxylon* lacks full vascularization of lateral appendages. Neither has a sporangium associated with a leaf, as occurs in members of the lycopsid clade and most probably in *Baragwanathia* (Hueber, 1992).

TIME OF APPEARANCE.—The occurrence of the genus *Baragwanathia* in the presumed late Silurian (Ludlow; Garratt *et al.*, 1984; Rickards, 2000) of Victoria, Australia suggests a possible pre-Devonian diversification of lycophytes. Zosterophylls also are recorded from these strata, but detailed descriptions are lacking (Tims and Chambers, 1974). Most other occurrences of late Silurian plant remains are much smaller in size and simpler in organization than either *Baragwanathia* or *Zosterophyllum* (Fig. 2). An interesting exception is the assemblage of zosterophylls and related plants from well-dated late Ludlow strata of Bathurst Island (Kotyk *et al.*, in prep.). These new forms support an early diversification of the lycophyte lineage.

POSSIBLE PRECURSORS.—Most polysporangiate plants (i.e. plants with branched sporophytes) with round to reniform sporangia referred to either as rhyniophyte?, cooksonioid, or rhyniophytoid (Taylor, 1988; Hueber, 1992) represent possible close relatives or precursors to the lycophytes but currently there are no obvious candidates. Outline drawings (Fig. 2) of several Late Silurian and Early Devonian rhyniophytoids show that these plants mostly are very small and exhibit variable sporangial shapes and modes of dehiscence. Their small size, simple construction, lack of anatomical preservation, and fragmentary preservation make more precise inference impossible, but those with globose-reniform sporangial construction may represent likely candidates for lycophyte precursors. A few larger forms with similar sporangial morphology have been recorded in pre-Devonian or earliest Devonian deposits, such as the specimen from the Ludlow of Bathurst Island (Basinger *et al.*, 1996; Kotyk, 1998) which resembles a pseudomonopodial *Cooksonia* or very simple *Zosterophyllum* (fig. 2 I).

Renalia, and some *Cooksonia* species, inferred to have arisen from within the plexus of latest Silurian-earliest Devonian rhyniophytoids (but their rela-

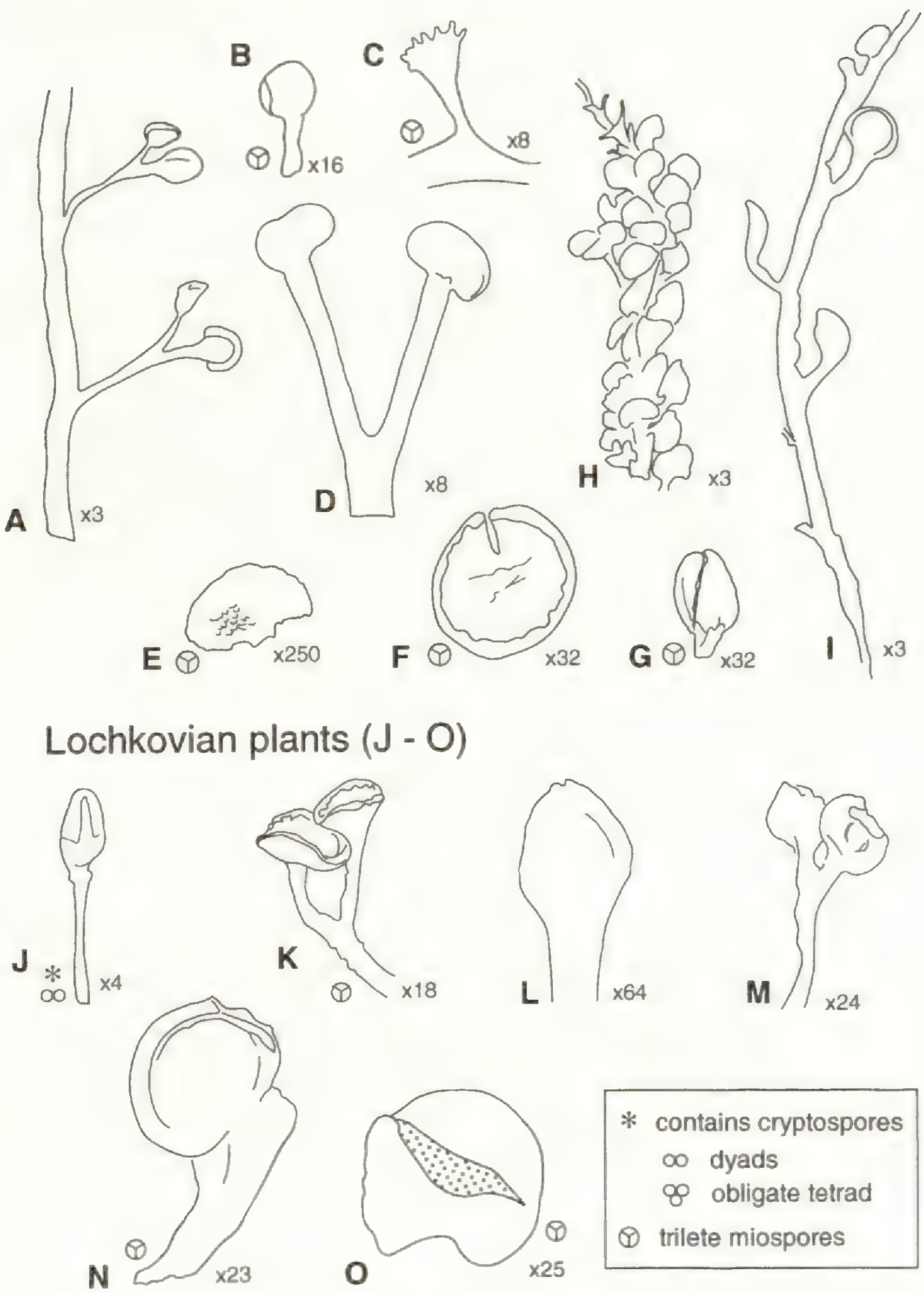

FIG. 2. Selected late Silurian and earliest Devonian meso- and megafossils. A—I) Pridoli plants. A) from Cai *et al.* (1993); B—D) from Fanning *et al.* (1991); E—G) from Edwards (1996). H) from Kotyk (1998). I) from Basinger *et al.* (1996). J—O). Lowermost Devonian plants from Edwards (1996).

tionship to these simple plants thus far is not extensively tested by cladistic analyses), also represent likely precursors. When *Renalia* and *Cooksonia* were included in the phylogenetic analyses of Kenrick and Crane, they formed part of the large basal polytomy of the polysporangiophytes and were interpreted as possible stem-lineage zosterophylls. Further investigation of these early plants is needed.

The present contribution notes major advances and addresses several areas of concern in regard to early lycophyte evolution and diversification, including current views of zosterophylls, early lycopsids, and evolution of major lycophyte structures.

Current Data on Zosterophylls

As a probable sister group(s) or stem group(s) to lycopsids, zosterophylls *sensu lato* (Fig. 3A–3C) may provide evidence of early evolutionary patterns within these lineages. However, presently recognized taxa, while demonstrating greater diversity of form and some new combinations of characters, do not alter conceptions of relationships between zosterophylls and lycopsids nor aid in clarifying ordinal or familial delimitation within zosterophylls. The two main zosterophyllopsid clades *sensu* Kenrick and Crane (1997) can be typified by two genera, *Sawdonia ornata* (Fig. 3A) and *Oricilla bilinearis* (Fig. 3C). Differences include sporangium orientation and presence/absence of emergences (multicellular appendages). The *Sawdonia* clade (Kenrick and Crane's Sawdoniaceae) exhibits more upright orientation of sporangia and emergences of varying morphology and arrangement, while the *Oricilla* clade (Kenrick and Crane's Gosslingiaceae) exhibits naked axes and sporangia stalks attached perpendicular to the axis. The *Zosterophyllum* depicted in Fig. 3B exhibits helically arranged sporangia and is most similar to those species of *Zosterophyllum* that form part of the basal polytomy in Kenrick and Crane's analyses. Beyond these two well- supported clades, we now have considerable new information about some of the more basal lineages, discussed next.

Plants which appear very similar to *Zosterophyllum* (Fig. 3B), except that sporangia are more scattered and borne on longer stalks than in many previously described *Zosterophyllum* species, are found in Pragian deposits of China and Bathurst Island, Canadian Arctic Archipelago (Kotyk, 1998). These do not quite fit existing generic or species delimitations, but as they become more completely known, they might aid in a better understanding of the genus *Zosterophyllum* or closely allied taxa. The separation of several *Zosterophyllum* species into separate clades in Kenrick and Crane's analysis is based mostly on symmetry of sporangial arrangement and presence/absence of circinate vernation. However, these characters are not easy to evaluate and their coding may not reflect that data about them are incomplete; most particularly, several taxa lacked anatomy. It is difficult to document the presence or (known) absence of circinate vernation (and true terminations). The character "symmetry of sporangial arrangement" may be difficult to ascertain because determining if sporangia are helically arranged or are subopposite in two rows is difficult in compression remains. A two-rowed pattern could be produced by a widely spaced, very simple helix coupled with twisting of either sporangial stalks or the axis (see Gerrienne, 1996b for an analysis of this feature). Current analyses indicate these new *Zosterophyllum* species and other *Zosterophyllum*-like plants may represent stem zosterophylls for which only a few distinguishing characters are present.

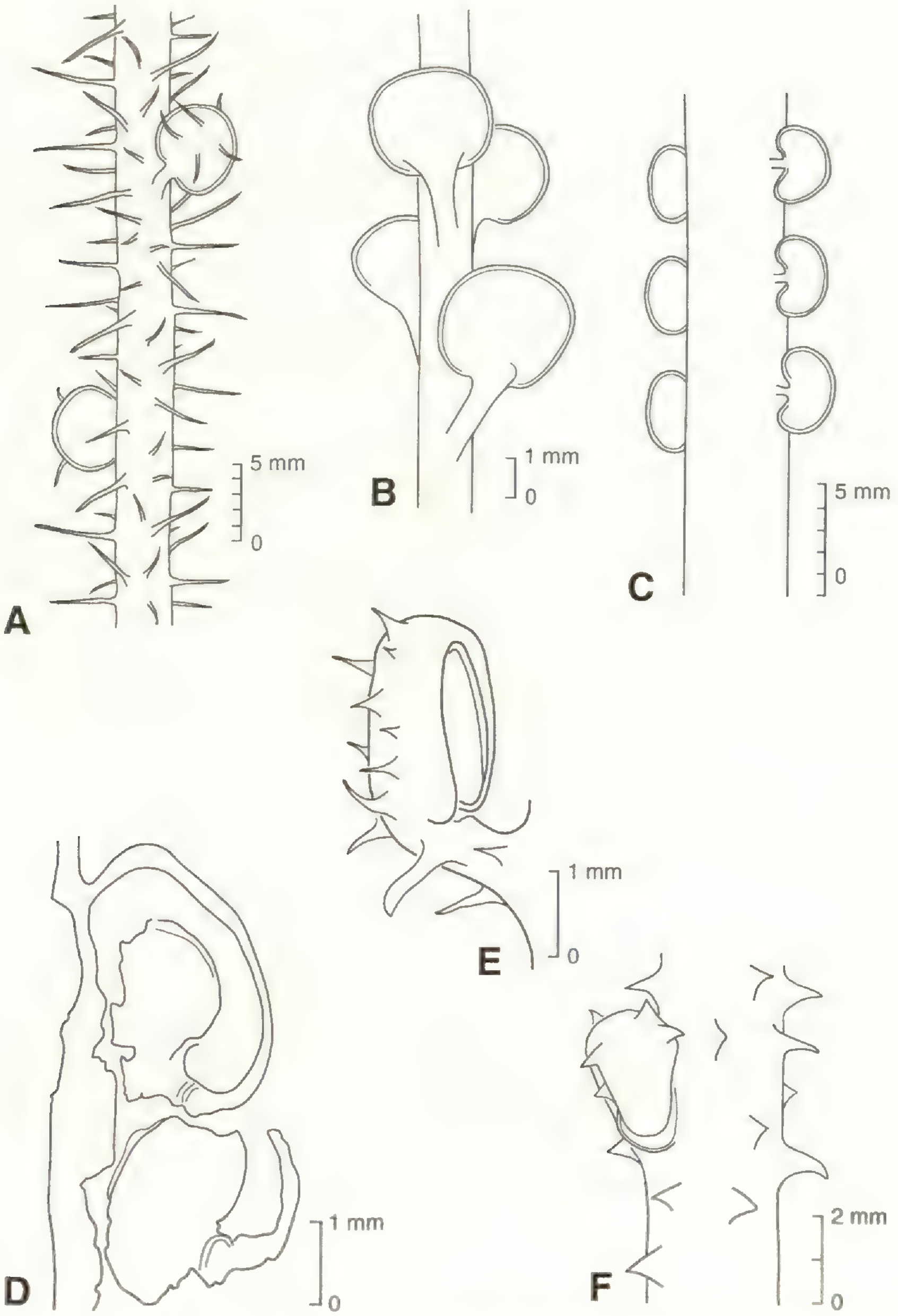

FIG. 3. Selected putative basal lycophytes or zosterophyllopsids. A, C) Representatives of Zosterophyllopsida *sensu* Kenrick and Crane. A) *Sawdonia ornata* (Dawson) Hueber, based on specimens from Gaspé. C) *Oricilla bilinearis* Gensel, redrawn from Gensel (1982). B) Depiction of a *Zosterophyllum* with helically arranged sporangia, a stem group taxon according to Kenrick and Crane (1997). D) *Gosferia curvata* Gerrienne from the Early Devonian of Belgium. Note thickening near base of sporangium. Redrawn from Gerrienne (1991). E) *Ensivalia deblondii* Gerrienne, a probable zosterophyllopsid from the Early Devonian of Belgium. F) *Faironella valentula* Gerrienne, affinity uncertain, from the Early Devonian of Belgium. Sporangium is borne inside a recurved, thickened spiny structure. E, F) redrawn from Gerrienne (1996).

Another possible basal taxon, *Gosferia curvata* Gerrienne (1991, 1999), from the Early Devonian of Belgium, is unique in having long, basipetally recurving stalks, each terminated by a reniform sporangium (Fig. 3D). The closest similarity of this plant is to some unidentified zosterophyll-like plants in China and Bathurst Island and to some Silurian-Pragian rhyniophyte-like plants. The occurrence of a swelling near the sporangium base where it attaches to the stalk also is of interest; similar swellings are known in some *Drepanophycus* species (Li and Edwards, 1995) and in *Kaulangiophyton* (Gensel, pers. obs.). What it represents in any of these plants is currently unknown (but see below).

Ensivalia deblondii Gerrienne (1996a) from the Early Devonian of Belgium has spiny, anisovalvate sporangia terminating stout stalks. The abaxial sporangial valve is enlarged and spiny, inside of which the more delicate, smooth adaxial valve rests (Fig. 3E). *Odonax borealis* Gerrienne (1996b), also from Belgium, and some unnamed zosterophylls from the Early Devonian of New Brunswick also exhibit modification of the sporangium/stalk in the form of enlargement of either stalk or distal sporangium valve and presence of emergences, features which perhaps help protect the sporangium.

Further evaluation of the variability of emergence types (see Gensel, 1992 and Kenrick and Crane, 1997 for examples) among zosterophylls is needed as well as, perhaps, clarification of the homologies of different types of emergences. For example, in *Nothia aphylla* (Lyon, 1964) El Saadawy and Lacey (1979), the so-called emergences are produced by raised areas of axial tissue (cortex and epidermis), resulting from expansion of existing cells, with apically located stomata (Kerp *et al.*, 2001). Are these structures truly emergences or do they represent short, discontinuous ridges of the stem? The unusual prismatic emergences of *Serrulacaulis* (Berry and Edwards, 1994) and those now known to occur in *Bathurstia* (Kotyk and Basinger, 2000) also add to diversity of those structures but do not provide any further tests of homology between emergences and microphylls.

Some Early Devonian plants in which sporangia are arranged in an obvious strobilus may relate to the lycophyte clade, either among zosterophylls or lycopsids. *Demersatheca*, based on only a few leafless fertile specimens from the Pragian of China, exhibits reniform sporangia deeply sunken into the axis (Fig. 4D) and forming tight strobili (Li and Edwards, 1996). Other plants with strobili include *Distichophytum* and *Bathurstia*, both attributed to the zosterophylls, and some un-named plants of uncertain affinity from Bathurst Island. This morphology blurs the distinction between zosterophyll and barinophyte to some extent.

Barinophytes are defined, according to Kenrick and Crane, by a very compact, unbranched strobilus, indehiscent sporangia, intrasporangial heterospory, and sporangia borne on a horizontally extended axis which wraps around the stem (their "clasping" sporangium orientation). However, their relationships are unclear as the Barinophytaceae form part of a polytomy that includes some major groups of zosterophylls in Kenrick and Crane's analysis. More data are needed about sporangium shape, attachment, and dehiscence in both the early strobilate plants and some plants usually attributed to barinophytes, such

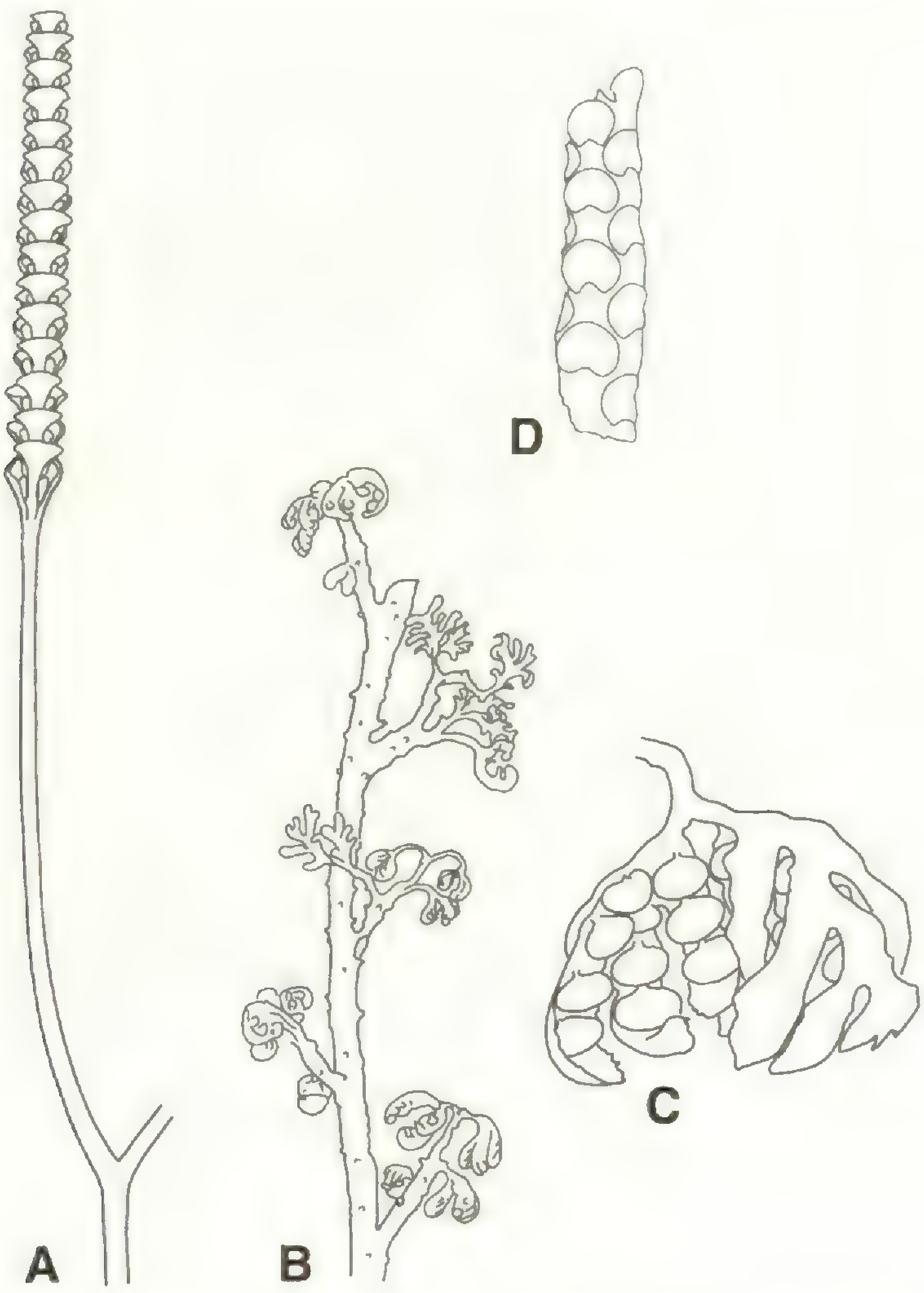

FIG. 4. Plants from the Pragian of China that may be related to lycophytes. A) *Adoketophyton subverticillatum* Li and Edwards with a leaf-like appendage subtending each sporangium. Redrawn from Li and Edwards (1992). B, C) *Eophyllophyton bellum* Hao, in which lobed leaf-like structures terminate lateral axes. Globose to reniform sporangia attach on the upper surface of the lobes. Redrawn from Hao and Beck (1993). D. *Demersatheca contigua* Li and Edwards, with sporangia sunken in the axes. Redrawn from Li and Edwards (1996).

as *Protobarinophyton*. *Faironella valentula* Gerrienne (1996a) is also of interest in this discussion as a possible link between zosterophylls and barinophytes. Its sporangia are attached to the inside of a curved appendage (Fig. 3F), and while affinities presently are uncertain, it thus bears some resemblance to Barinophytaceae.

Adoketophyton, a genus described from the Pragian of China by Li and Edwards (1992), demonstrates fertile regions with leaf-like structures that bear sporangia in their axils (Fig. 4A). Crane and Kenrick (1997) suggest the leaf-like structures might represent sterilized sporangia that became modified as a "leaf" as a result of changes in developmental processes such as multiplication and co-option. Another plant from the Pragian of China, *Eophyllophyton bellum* (Hao) Hao and Beck (1991) has the habit of a typical Early Devonian plant, but bears lobed, laminate leaf-like structures (Fig. 4B, 4C). Whereas this plant is at the base of the euphyllophyte clade of Kenrick and Crane, largely because

FIG. 5. Line drawing of *Drepanophycus qujingensis* Li and Edwards. Note cauline sporangia not associated with leaves. Redrawn from Li and Edwards (1995).

of its centrarch protostele, its globose-reniform sporangia are located adaxially on leaves and its tracheids possess G-type wall thickenings like those present in many zosterophylls and stem-lycophytes. In our opinion, this plant possesses an odd suite of characters for basal euphyllophytes and it is perhaps more appropriately considered in the context of zosterophylls or lycopsids. Both *Adoketophyton* and *Eophyllophyton* thus provide idiosyncratic evidence of the development of laminate appendages, but little information on their homologies.

PRE-LYCOPSIDS

This term has been applied to plants with many lycopsid characters, but lacking the consistent association of sporangium with a leaf or lacking fully vascularized leaves. Genera included are *Drepanophycus, Kaulangiophyton, Asteroxylon,* and in some instances, *Baragwanathia* (for another interpretation, see Hueber, 1992). They form a sister clade to other lycopsids in Kenrick and Crane's analysis and are treated there as a stem-based group of Lycopsida, the Drepanophycales. However, some aspects of their structure remain controversial, and re-evaluation of these features may influence their position relative to lycopsids or the interpretation of their evolutionary significance.

Based on current knowledge of *Drepanophycus,* the following appears certain: two species of *Drepanophycus, D. qujingensis* Li and Edwards and the type species *D. spinaeformis* (Goeppert) Li, Hotton and Hueber, exhibit both rhizomatous and aerial axes covered with vascularized microphyllous leaves and cauline sporangia (Fig. 5; Li and Edwards, 1995; Li *et al.*, 2000). Among

vegetative remains assigned to *Drepanophycus*, leaf morphology varies considerably, from long and falcate to short and thorn-like. It has been suggested that the axes with the former represent aerial regions, those with the latter rhizomatous axes. Sporangia borne on comparatively short stalks (usually shorter than leaves) are interspersed among the microphyllous leaves, with no apparent association with them.

Kaulangiophyon akantha exhibits a similar growth habit to that of *Drepanophycus*, but with all axes covered in stout, thorn-like emergences (Gensel *et al.*, 1969). Studies are underway to test assertions that *Kaulangiophyton* is very similar to or congeneric with *Drepanophycus* (Kasper, 1972; Schweitzer and Giesen, 1980), or that *Kaulangiophyton* has sporangia associated with leaves (Hueber, 1992). Careful examination of original Maine collections and new material from New Brunswick shows no indication of vascularization in the emergences. Sporangia were originally described as cauline, terminating a moderately long stalk and extending beyond emergences (Gensel *et al.*, 1969). Hueber (1992) noted features of the sporangial stalk that led to his hypothesis that a leaf extends beneath the sporangium but is obscured by the sporangium and thus is not visible on impression remains. We note that the stalks of some sporangia attach to a thickened pad or ring of coalified material that itself is borne on an axial protrusion. It resembles the pad at the base of sporangia in *Stockmansella* and *Huvenia*. A similar thickening may occur in *Drepanophycus qujingensis*. However, in contrast to Hueber's ideas, no extra appendage, emergence, or leaf lamina has been demonstrated in attachment to this thickening in *Kaulangiophyton*. Additional information on these and other characters of *Kaulangiophyton* and assessment of relationships will be presented in a later paper.

Rooting Structures in Early Lycophytes

Information about rooting structures, other than rhizoids, in basal lycophytes is increasing, being recorded for the zosterophylls *Zosterophyllum, Bathurstia, Crenaticaulis* (Gensel *et al.*, 2001), and *Hsua* (Li,1992), for *Drepanophycus* (Rayner, 1984; Li and Edwards, 1995; Gensel *et al.*, 2001) and for the rhyniacean *Stockmansella* (*Taeniocrada*) *langii* Fairon-Demaret (1985) by Schweitzer (1980). Two patterns are recognized by Gensel *et al.* (2001) and may correlate with habit. The first pattern occurs in tufted forms, such as some *Zosterophyllum* species, which produce downward trending axes from a central region that appear root-like. Upward trending axes departing from the same point are interpreted as shoots. In the other pattern, slender smooth axes depart from the rhizomatous regions, and in some cases divide to varying degrees. These frequently lie across, not on, the bedding plane. Also, they are sometimes produced during the formation of K-branches as in *Bathurstia* and *Drepanophycus* (Gensel *et al.*, 2001). In these plants, an axis departs, then forks. One of the resultant axes is oriented towards the rhizome apex and appears identical to shoots and the other, lacking either emergences or leaves and directed away from the apex, appears root-like (Figs. 6A, 6B). Based on

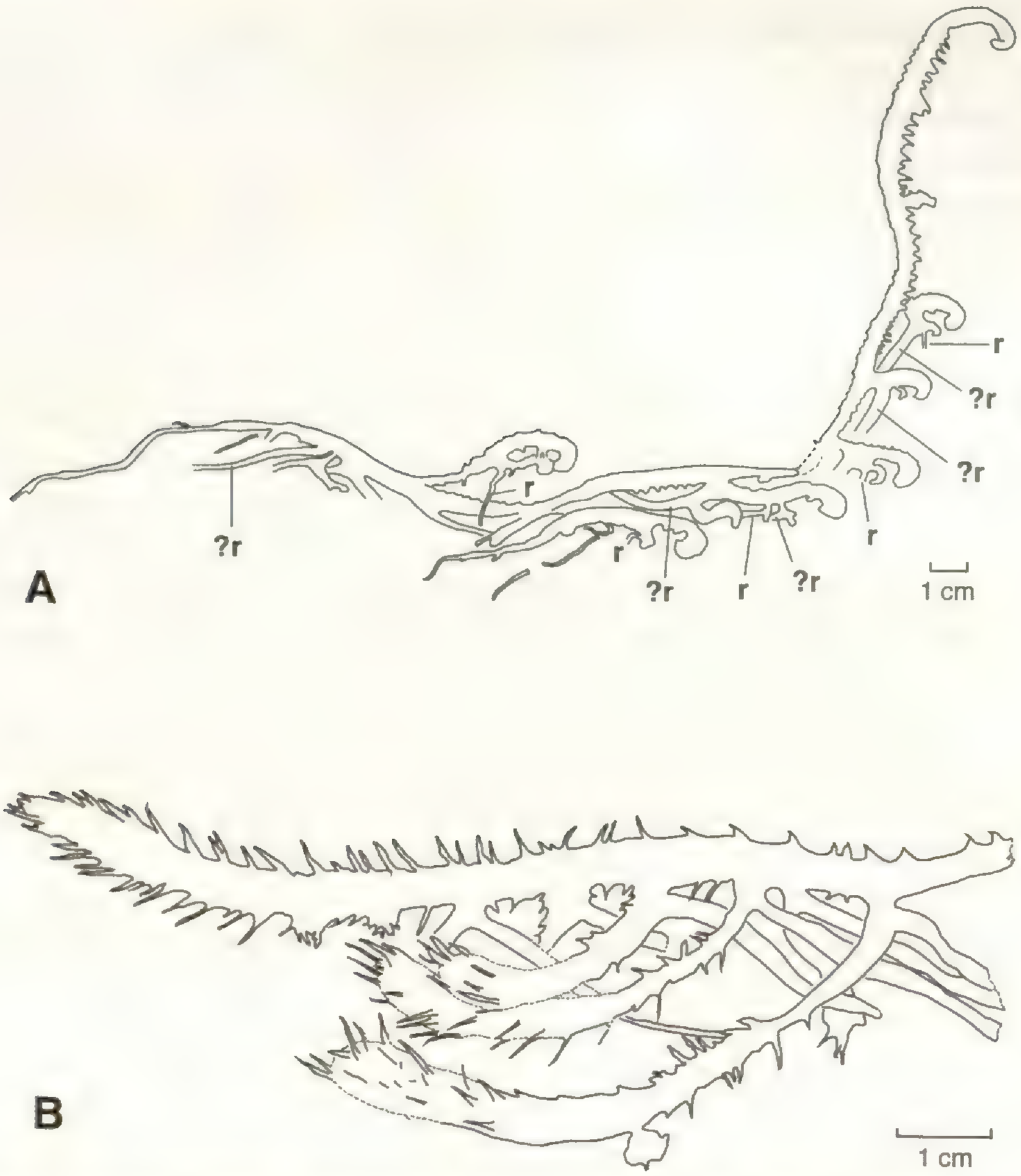

FIG. 6. A) Line drawing of the zosterophyll *Bathurstia denticulata* (Hueber) Kotyk and Basinger, showing numerous K-branches in which one portion is a rooting structure and the other a shoot-like structure, frequently in crozier form. B) Line drawing of *Drepanophycus* from the Pragian of Bathurst Island, showing several K-branches in which one part is a shoot and the other a smooth, root-like organ. r = rooting organs. Redrawn from Kotyk (1998), with permission and Gensel et al. (2001).

current evidence, these rooting structures appear to be similar and perhaps homologous to shoots. Gensel *et al.* (2001) postulate that among lycopsids, and perhaps most non-seed plants, roots arose via a dichotomy of the shoot system, with one apex transformed into a root apex. This condition occurred over time progressively earlier in ontogeny, until the root arose in the embryo. The position of the root primordium lateral to a shoot apex in many pteridophyte embryos may be significant in this regard. Furthermore, roots and shoots thus are homologous structures. None of the rooting structures among zosterophylls or lycopods have any kind of emergences or leaves on them except perhaps some fine hairs on those in *Crenaticaulis*. These differ from any ornament on aerial axes. Thus, part of the transformation to a root apex may have been to lose any potential to develop leaves or emergences.

Protolepidodendrales (Protolepidodendraceae, Haskinsiaceae, and Archaeosigillariaceae): Early Lycopsid Diversification

Following the Late Silurian-Early Devonian establishment of zosterophyllopsids and lycopsids, diversification of lycopsids is evidenced by abundant and widespread remains of lycopsids with distinctive anatomical and morphological features. Many of these form a group currently recognized as the Protolepidodendrales. These plants were prominent in Middle to early Late Devonian times and show more advanced and variable leaf morphology and vascular anatomy than pre-lycopsids. Sporangia where known are epiphyllous. Two families and a satellite taxon (Thomas and Brack-Hanes, 1984) are recognized within protolepidodendralean lycopsids.

The Protolepidodendraceae are well known from several parts of the world in Middle Devonian times. They consisted of lycopsids with helically arranged, forked leaves, and globose to elongate sporangia located on the adaxial surface of the leaf. Their characteristic leaves, which are divided into three (*Colpodexylon, Minarodendron*) or five or more (*Leclercqia*) segments, are the most striking features of the plants. In many of these taxa, sporophylls resemble vegetative leaves. Sporangia in *Leclercqia* attach by a single discrete layer of cells to the leaf just proximal to its division, and the free part of the sporangium is directed towards the axis. A single line of dehiscence extends the length of the sporangium. Mode of sporangial attachment in other protolepidodendrids is not as clear, but they apparently lack stalks. Permineralized remains of some of these plants show a solid xylem column with several narrow ridges of protoxylem around the periphery (*Leclercqia, Minarodendron*), resulting in a shallowly ribbed protostele, or a more deeply lobed xylem strand (*Colpodexylon*). *Leclercqia* is notable for being a ligulate, homosporous taxon (Grierson and Bonamo, 1979). Berry (1996) summarized the considerable new data and major features of these plants and other representatives of the Protolepidodendrales (Table 1).

Pre-Middle Devonian Protolepidodendraceae have been found. Kasper and Forbes (1979) recorded *Leclercqia* sp. from the late Early to Early Middle Devonian of Maine and Kasper (1977) noted the presence of a new species of *Leclercqia* in the Emsian of New Brunswick. In both of these occurrences, axes bear five-segmented leaves, but all segments are oriented in the same direction (Fig. 7B) rather than being three-dimensionally arrayed as in *L. complexa* Banks, Bonamo and Grierson. At the time of recording them, the age determinations of sediments were uncertain. Since then, dispersed spore data indicate the New Brunswick sediments are undoubtedly Emsian. Recent collections from these Emsian New Brunswick outcrops by Gensel document a second occurrence of the genus, these plants being very similar in leaf morphology and sporangial attachment to *L. complexa* (Fig. 7A), differing mainly in their smaller size.

Remains of a plant with leaves possessing a total of nine segments also have been found in the Emsian of New Brunswick. In these forms, four segments are oriented in an upwardly directed, flattened lamina to each side of a long,

TABLE 1. Synopsis of protolepidodendralean lycopsids.

PROTOLEPIDODENDRALES

- Especially abundant in Middle and early Late Devonian but some occurrences in Emsian (late Early Devonian-shown as asterisk below)
- Plants with dichotomously? branched axes, probably rhizomatous, creeping habit, no secondary tissues, no leaf scars (leaves persistent); stems protostelic, exarch, xylem with bordered pitted tracheids; leaves inserted in patterns resembling helices or pseudowhorls, sporophylls more or less identical to vegetative leaves, sporangia on adaxial surface of leaf.

PROTOLEPIDODENDRACEAE

Leclercqia* Banks, Bonamo et Grierson 1972
Minarodendron Li 1990
Colpodexylon Banks 1944
Protolepidodendron Kräusel *et* Weyland 1932
Leaves divided into up to 9 narrow segments; planate or three-dimensional.
Sporangia elongate ovoid; homosporous where known.
Protostele either lobed (*Colpodexylon*) or denticulate.

HASKINSIACEAE

Haskinsia* Grierson *et* Banks 1983 emend Berry et Edwards 1996
Artschaliphyton Senkevich 1971
Leaves petiolate; blade lanceolate, sagittate or hastate.
Sporangia globose to obovoid; spores unknown.
Protostele denticulate.

ARCHAEOSIGILLARIACEAE (satellite taxon)

Archaeosigillaria (Göppert) Kidston 1901
Gilboaphyton Arnold 1937 *emend* Berry et Edwards 1997
Leaves laminar, with a pair of prominent, lateral, distally oriented 'teeth'; "inflated" hexagonal leaf bases surrounded by subepidermal thickenings.
Fertile structures unknown.
Protostele lobed.

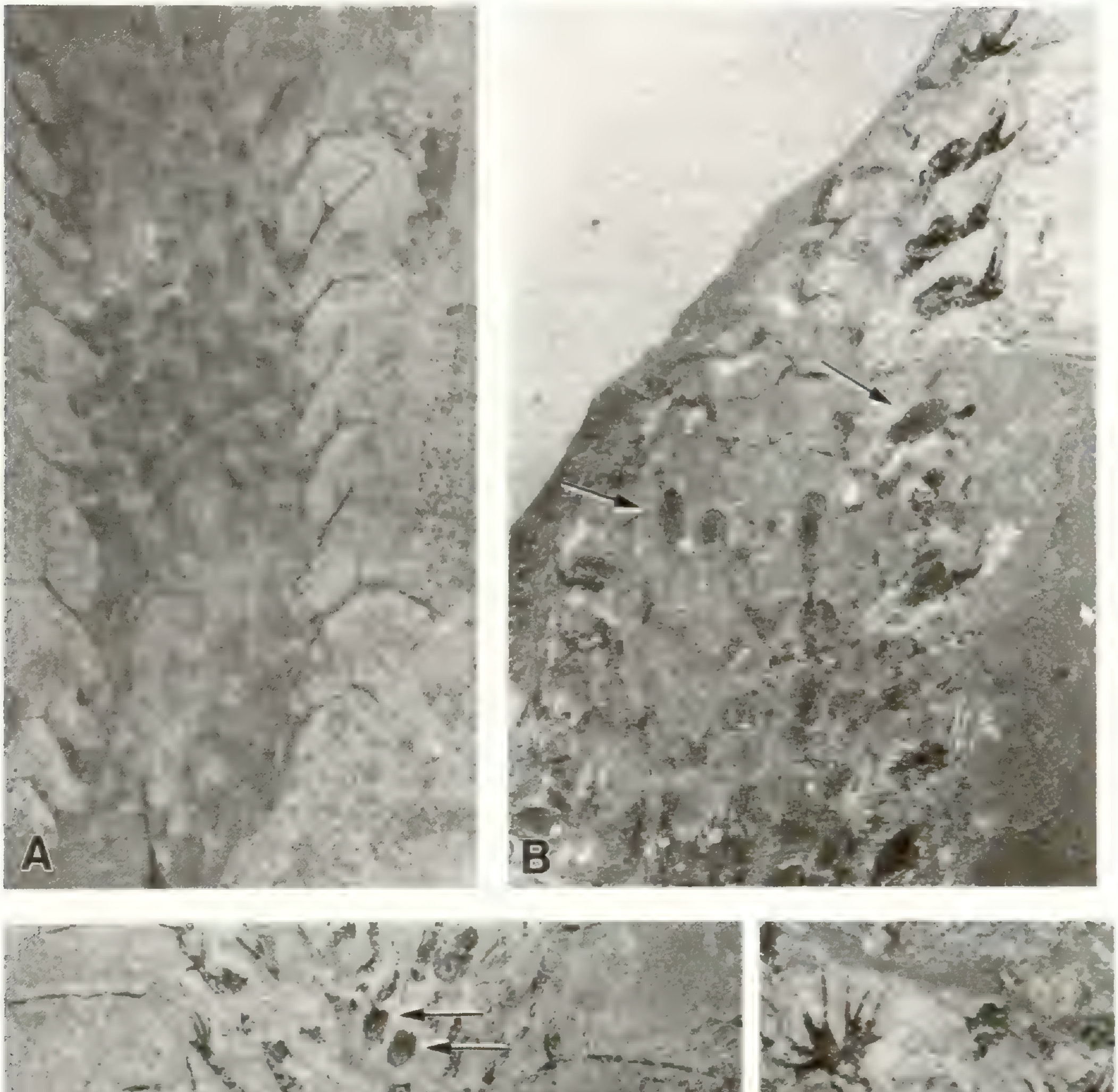

FIG. 7. Illustrations of Protolepidodendraceae from the Emsian of New Brunswick. A) *Leclercqia* collected by Gensel. This is most similar to *L. complexa*, 4X. B) *Leclercqia* sp. described in abstract by Kasper (1974). Oval bodies represent sporangia (arrows). 3.6X. C) New leclercqioid plant with 8- segmented leaf. Note long distal segment, upward trending lateral ones in side view on upper left, an imprint of a sporangium associated with leaf between arrowheads to lower right, other sporangia at arrows, 2.4X. D. New leclercqioid plant with 9-segmented leaf, lateral leaf segments in surface view, 4X.

central, horizontal to downward- extended one (Figs. 7C, 7D). Elongate sporangia attach, perhaps at one end, to the upper surface of the undivided portion of the leaf (Fig. 7C, arrows). Another genus with segmented leaves, *Cervicornus wenshanensis,* was reported from the Pragian of Yunnan, China (Li and Hueber, 2000). This genus has leaves that are divided into two groups of four (eight segments total) segments, arrayed three-dimensionally. Although lacking important diagnostic evidence such as sporangia and anatomy, *Cervicornis* may be the oldest record of Protolepidodendraceae. Thus, more diversification of this group prior to the Middle Devonian is evident. In terms of younger examples, a lycopsid of similar size as all examples of Protolepidodendraceae, and superficially identical in appearance, has been reported from Lower Carboniferous sediments of Argentina. *Frenguellia* Arrondo *et al.* (1991) has leaves with a long median segment, and two pairs of shorter opposite lateral tips, two of which point backward towards the stem. Sporangia are borne on unmodified leaves. However, we are concerned about the lack of conclusive and direct evidence for the present dating of these fossils; they may be substantially older.

A second protolepidodendralean type is represented by the Haskinsiaceae (Figs. 8A–8C). This family differs from the Protolepidodendraceae in having more laminate, hastate or sagittate leaves and perhaps in sporangium shape. Leaf morphology varies less within this group than in Protolepidodendraceae. Epiphyllous, globose or flattened ellipsoidal sporangia have been demonstrated in two species of *Haskinsia* from Venezuela, although mode of dehiscence and spores are unknown (Berry and Edwards 1996). *Haskinsia* from New York State has a primary xylem column with peripheral ridges of protoxylem similar to those of *Leclercqia* and *Minarodendron* (Grierson and Banks 1983).

Archaeosigillariaceae, including the genera *Archaeosigillaria* and *Gilboaphyton,* are sometimes regarded as members of the Protolepidodendrales but fertile examples are lacking. *Archaeosigillaria* has recently been restricted to the type specimen (Berry and Edwards 1997). Archaeosigillariaceae *sensu* Berry and Edwards (1997) includes Middle-Late Devonian plants which have a hexagonal pattern of swollen leaf bases on the stem surface, and permanent lanceolate (more or less hastate) leaves (Figs. 8E, 8F). A permineralized specimen of *Gilboaphyton goldringiae* Arnold from Gilboa (Grierson and Banks 1963, Pl. 37, figs 4, 7) demonstrates a protostele with approximately eight peripheral lobes in transverse section. Specimens of *Gilboaphyton griersonii* from Venezuela and New York State have similar leaf bases, and Berry and Edwards (1997) were able to infer the presence of elongate-oval, possibly parenchymatous, regions beneath each leaf base (Fig. 8D). These are suggestive of possible greater differentiation of stem tissue and may represent a prototypical form for one or more types of parenchyma/aerenchyma (e.g. parichnos) associated with leaf traces found in Late Devonian and Carboniferous lycopsids.

Among the Protolepidodendrales, where xylem anatomy is known, secondary wall pitting patterns include simple and bordered pits as well as annular and scalariform pitting. *Minarodendron* tracheids also exhibit pitlet sheets which resemble to some extent pitting found in younger lycopsids, termed

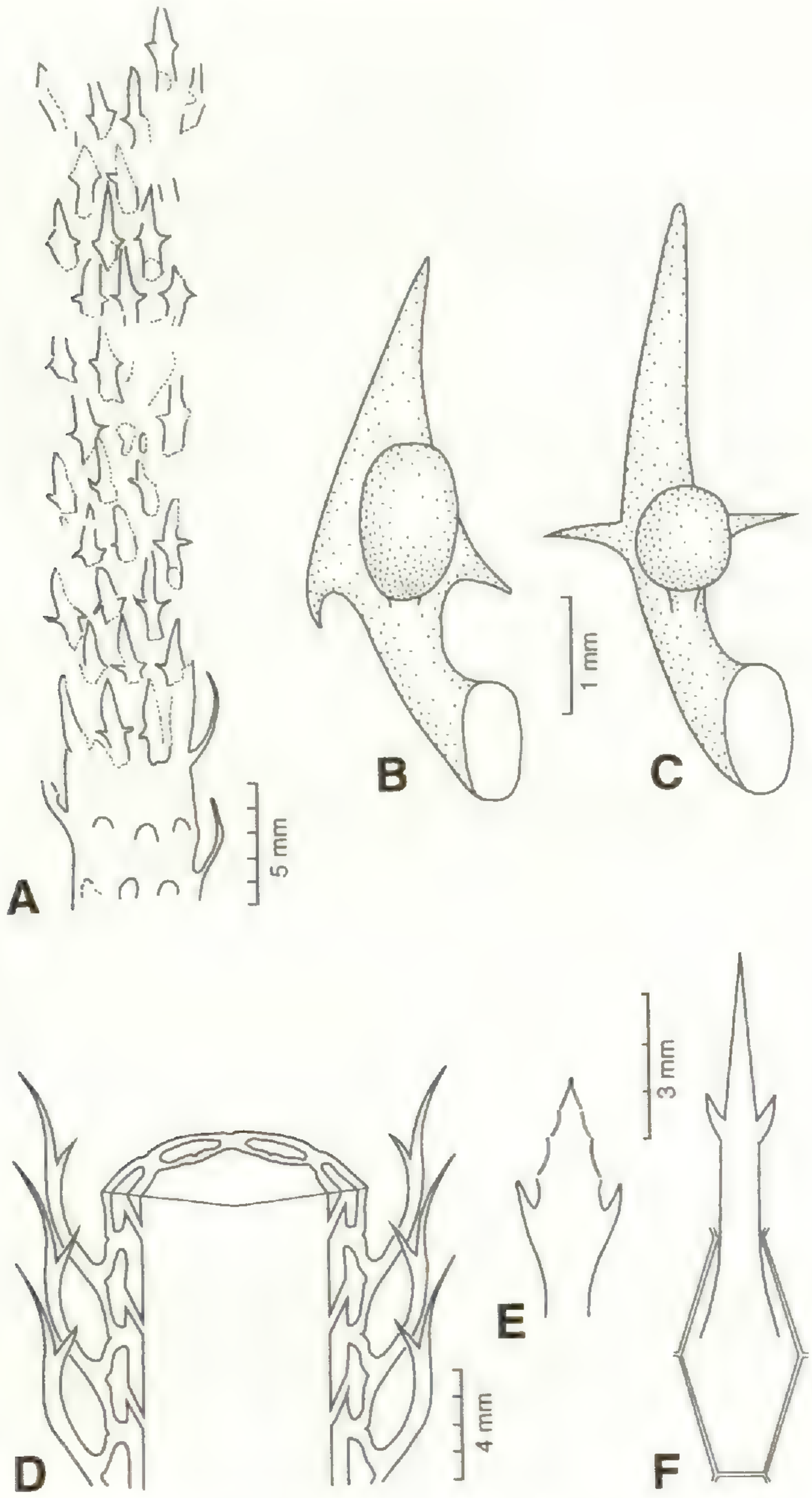

FIG. 8. Haskinsiaceae and Archaeosigillariaceae. A) *Haskinsia*- line drawing of sterile specimen of *H. hastata* from Venezuela. B) Fertile leaf and sporangium of *H. sagittata* Edwards and Benedetto emend. Berry and Edwards fromVenezuela. C) Fertile leaf and sporangium of *H. hastata*. D, F) *Gilboaphyton griersonii* from the Devonian of Venezuela. E) *Gilboaphyton goldringiae* from New York State. A—C) Redrawn from Berry and Edwards (1996), D—F) redrawn from Berry and Edwards (1997).

Williamson's striations or fimbrils. Fertile examples, where known, were apparently homosporous. No basal regions or rooting structures have been described. A ligule has been documented only in *Leclercqia*.

Few protolepidodendralean taxa were included in cladistic studies largely because many of them are not completely enough known. *Leclercqia* and *Minarodendron*, the most completely-known examples, consistently form a clade that is sister, or paraphyletic, to Selaginellaceae or as part of a polytomy with

Selaginellaceae and the rhizomorphic lycopsids (Kenrick and Crane, 1997; Bateman *et al.*, 1992). Further, earlier putative lycopsids such as *Drepanophycus, Asteroxylon,* and *Baragwanathia* usually are sister to all of these clades. Most troubling in Protolepidodendrales is our current lack of knowledge about their rooting structures. This feature has the potential to reveal crucial information about relationships of the Protolepidodendrales to other groups, including the arboreous forms. The lack of well-preserved rooting structures is worrisome in the context of the large amount of above-ground material known and the excellence of some of the descriptions. One possible implication is that these plants may not have had specialized rooting structures. Alternatively, rooting structures may have existed, but were not preserved in the same horizons where the stems occur.

Innovations in Growth Habit, Rooting, and Reproductive Structures in Devonian Lycopsids

Until recently, the Protolepidodendrales, or certain less well-known remains, were the most likely group from which the Late Devonian and Carboniferous arborescent, ligulate, heterosporous lycopsids may have arisen. A crucial difference between Protolepidodendrales and aborescent forms however, is that the latter have a bipolar shoot system and erect growth habit. New discoveries from China have provided important evidence about changes in growth habit and rooting structures among Devonian lycopsids.

Small Tree-Like Lycopsids of the Middle Devonian.—The earliest examples of true bipolar growth, resulting in upright growth of a trunk and downward growth of a dichotomous rooting system, are to be found in the late Middle Devonian (Givetian) and earliest Late Devonian (Frasnian) of China. The two plants in question are *Longostachys* (Cai and Chen, 1996) and *Chamaedendron* (Schweitzer and Li, 1996). Both are small in height (0.5–1.5m), having a slender trunk that branches isodichotomously at the top to form a crown of narrow branches and terminal cones, and at the base to form a downward-pointing, conical- shaped, rooting system which divides into four major segments (Fig. 9). Roots may dichotomize further but lack any sign of attched rootlets or stigmarian-type rootlet scars.

Longostachys is the larger of the two plants and has secondary growth in both the trunk and the rooting system. Sterile leaves are long and narrow, with spiny margins. Fertile leaves are spoon-shaped (the bowl containing the sporangium nearest the axis), and also have spiny margins (Fig. 9). Both of these plants are interesting because they have major features characteristic of rhizomorphic lycopsids (habit, dichotomous rooting system, bipolar growth) but lack rootlets.

Late Devonian Rooting Structures.—The famous 'Naples tree' (*Lepidosigillaria whitei*) from the Late Devonian of New York State has previously been interpreted to have a swollen base covered in stigmarian rootlets (White, 1907; Pigg, 1992, 2001). This specimen is poorly preserved, and suffering pyrite de-

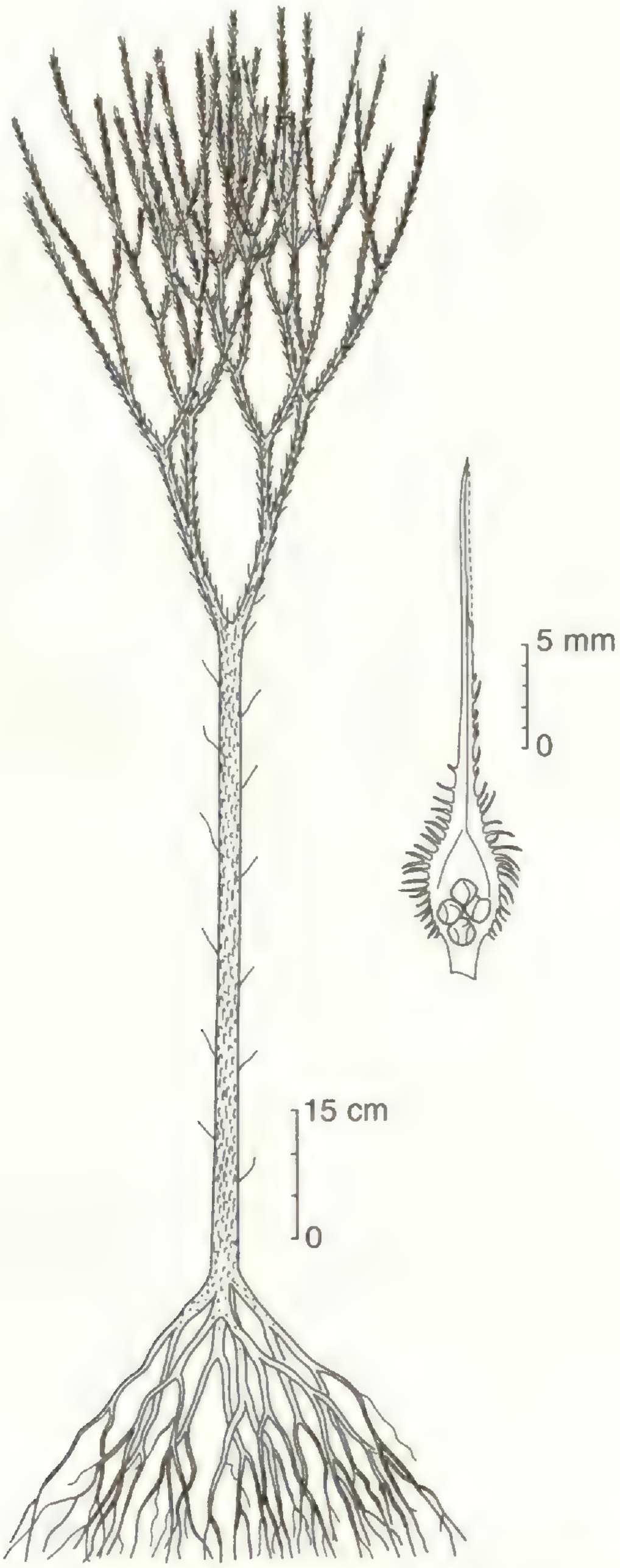

FIG. 9. Reconstruction of *Longostachys*, showing the four-parted rooting structure and leafy stems, and (inset) a single sporophyll. After Cai and Chen 1996, with permission.

cay, and the presence of rootlets and rootlet scars can no longer be verified. The poor quality of White's photographs showing the basal region of the plant compared to his other photographs of the upper trunk would suggest it was no better preserved when he worked on it. Thus we cannot accept this specimen as showing the earliest example of a lycopsid rhizomorph. Earlier Middle Devonian stumps named *Eospermatopteris* (Goldring 1924) which several peo-

ple considered to be possible lycopsid bases (as noted by Pigg, 1992), are far more likely to be cladoxylalean in affinity (Boyer and Matten 1996; Berry and Fairon-Demaret in progress). *Clevelandodendron,* from the Late Devonian (Famennian) Cleveland Shale, has an isoetalean habit but again stigmarian rootlets were not demonstrated (Chitaley and Pigg, 1996). Branched rooting structures are reported, but not described in any detail, for the Upper Devonian *Cyclostigma kiltorkense* (Johnson, 1913). *Leptophloeum* from the Upper Devonian (Famennian) of South Africa has a bulbous base from which many thick dichotomous roots emerge all around (Gess and Hiller 1995). These roots are up to 10 mm wide in comparison with the 45 mm bulbous base width and seem too large to be rootlets. This suggests an absence of rootlets in this plant. Lastly, cormose lycopsid plant bases, some with attached rootlets, have been reported from the Upper Devonian (?late Famennian age) Red Hill, Pennsylvania deposits (Cressler, 1998, 1999; Scheckler *et al.*, 1999).

The previous earliest western occurrence of an undisputed lycopsid rhizomorph (Pigg, 2001) is the report by Jennings et al. (1983) of *Protostigmaria eggertiana* from the Mississippian of Virginia, USA. Chinese occurrences include *Stigmaria rugulosa* Gothan from the Upper Devonian Wutung Formation near Nanjing (Gu & Zhi 1974). Berry and Wang Yi collected dichotomous stigmarian roots with characteristic rootlet scars from Nanshan Quarry, in Jiangsu Province, also from the Wutung Formation (unpublished) where other fossils included *Eviostachya* and *Hamatophyton* (Li, Cai and Wang 1995).

The facts, as outlined above, demonstrate that the emergence of the lycopsid rhizomorph remains a mystery. According to Rothwell and Erwin (1985) 'there now can be little doubt that the rooting structures of all rhizomorphic lycophytes are shoot systems modified for rooting' and implied rootlets therefore were homologous to leaves (p. 95). Bateman *et al.* (1992) however recognize that the stigmarian rhizomorph 'is a shoot-like developmental system', but prefer to regard it as a unique organ reflecting limited developmental options within the arborescent lycopsid bauplan'. The new evidence from China suggests that the earliest lycopsids with bipolar growth, although possessing a dichotomous, downwards-pointing rooting system, did not possess stigmarian rootlets, supporting Bateman et al.'s hypothesis. Such rootlets are not found until the uppermost Devonian in China and either uppermost Devonian or Lower Carboniferous in the U.S.A.

By the time tree-shaped lycopsids appeared in the Middle Devonian, leaves were well developed as structures, and it seems inconceivable that leaves might have been involved in the development of a downward-directed rhizomorph as implied by Rothwell and Erwin's hypothesis. If the K branching recognized above in zosterophylls and pre-lycopsids (Gensel *et al.*, 2001) does in fact represent the earliest stages of bipolar growth in the Lycophytina (*sensu* Kenrick and Crane 1997) we can propose homologies between structures in zosterophylls and arborescent lycopsids (Fig. 10). In some zosterophylls and in *Drepanophycus,* K-branching occurs, the posterior (or downwards)-directed branch being naked and the apically (or upwards)-directed branch bearing enations or leaves. The two branches are homologous structures, deriving from a

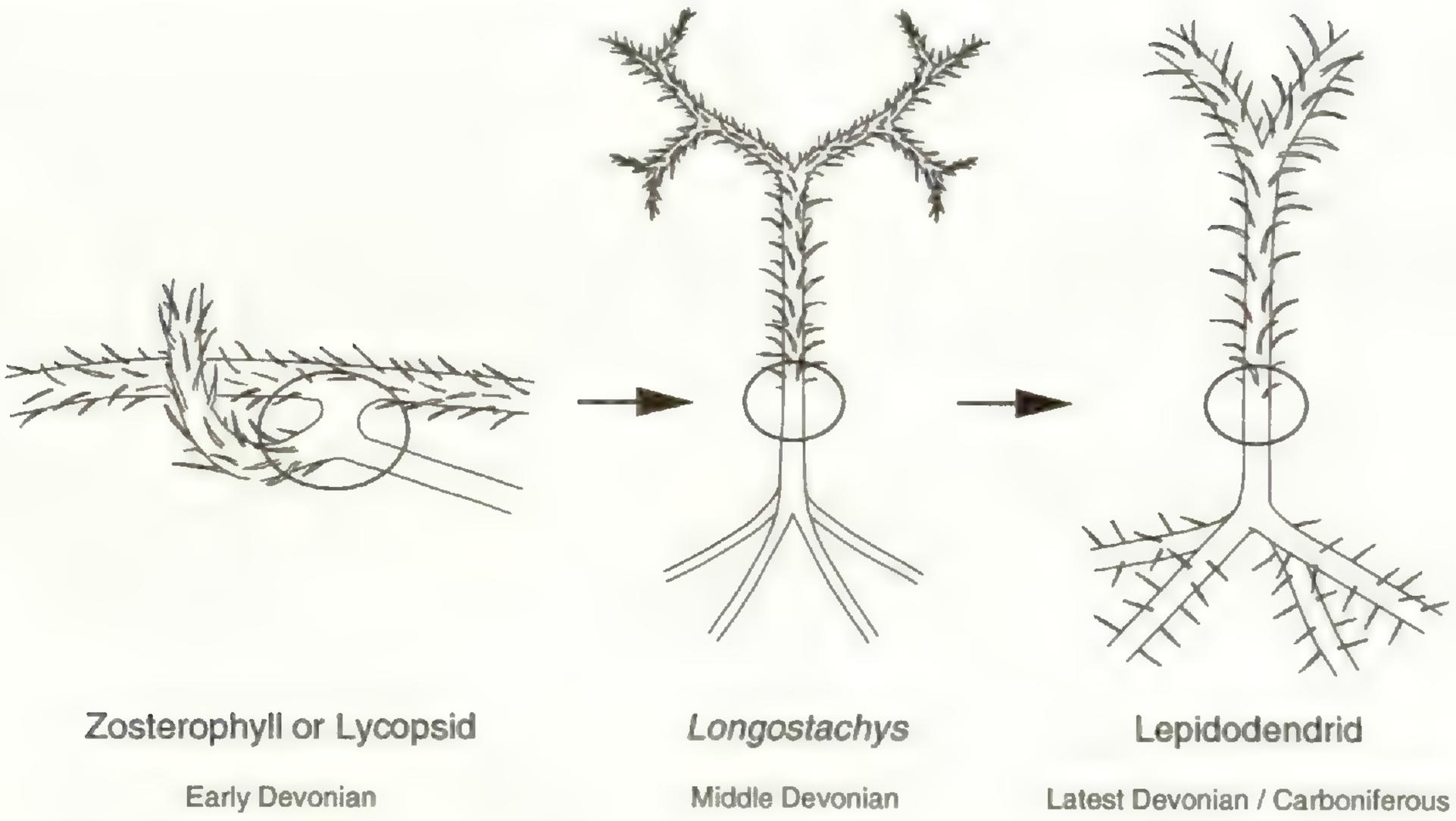

FIG. 10. Hypothesis of homology of rooting structures between creeping early lycophytes (zosterophyll or lycopsid), *Longostachys*, and a Carboniferous arboreous lepidodendrid. Shoot and rooting structures are homologous, being derived by a branching event. Early rooting structures lack lateral appendages; rootlets arose later, possibly in early arboreous forms. See text for further explanation.

regular dichotomous branch as found in all zosterophylls, but the posterior-directed branch having developed the characters of roots and loss of enations/leaves. In the earliest pseudobipolar lycopsids (Middle Devonian of China) the K branching occurs precociously at the initial growth of the sporophyte, causing upward growth of the bifurcate, leafy aerial axes and downward growth of the bifurcate, naked rooting system. Final development of the arborescent lycopsid body plan occurs when rootlets evolve *de novo* on the rooting system to increase efficiency.

INNOVATIONS IN FERTILE STRUCTURES AMONG MIDDLE AND LATE DEVONIAN LYCOPSIDS.—Middle Devonian Protolepidodendrales bear spores in single adaxial sporangia where known. Characteristically the fertile leaves are unmodified compared with the sterile ones. Often this means that the sporangia are borne more or less exposed on narrow leaf pedicels before the leaf forks (e.g. *Leclercqia, Colpodexylon*). In Haskinsiaceae, however, the laminate nature of the leaf and its upcurved pedicel (Figs. 8B, C) means that the sporangia are slightly more protected by the leaf (Berry and Edwards 1996). In those Protolepidodendrales where spores are known, the plants are homosporous. Sporophylls are often found concentrated into areas of the axes, but show no closer insertion nor increased overlap to areas of sterile leaves.

In *Chamaedendron,* leaves are slightly broader near their base, but in *Longostachys* sterile leaves are narrow and fertile leaves are distinctly enlarged near their base in the area of attachment of the sporangia (Fig. 9), the shape being suggestive of an upturned spoon. The leaf margins of these Chinese lycopsids are spiny. The enlarged bases of the sporophylls are curved around

sporangia which contain large megaspores, demonstrating that the plant is probably heterosporous (microsporangia are unknown). Leaves are not well enough preserved to know for certain if ligules were present or not. In these small, bipolar, tree-shaped lycopsids the sporophylls are crowded closer together forming distinctive fertile regions, and individual leaves overlap to some extent. Such fertile regions are therefore intermediate between the lax protolepidendrid fertile areas and the compact cones of many more advanced lycopsids.

Lycopsid strobili from Kazakhstan, correlated to the upper part of the Givetian on the basis of plant fossils, are reported to contain a random mixture of mega- and micro-sporangia (Senkevich et al., 1993). These demonstrate an early record of compaction of sporophylls to form a tight, protective strobilus in lycopsids.

A new probably arborescent lycopsid from the uppermost Devonian of Wuxi, China (Berry, Wang, and Cai, in prep), with branches slightly wider than those of *Longostachys,* demonstrates the earliest known occurrence of a lycopsid that has distinct micro- and megasporangiate cone-like structures. Megasporangiate 'strobili' contain leaves similar to those of *Longostachys* (but up to 90 mm long) whereas the microsporangiate strobili contain closely packed sporophylls that are much narrower. The megasporangiate strobili of this lycopsid are not terminal, but occur at dichotomies on the distal axes. Thus some modification and compaction of sporophylls is well underway prior to the end of the Devonian.

Conclusions

Important new information has been obtained in recent years about the early history of lycophytes, particularly concerning anatomy and morphology of shoots, the occurrence of rooting structures, and greater diversification and differentiation of fertile regions. Recent broadly based phylogenetic analyses of vascular plants present interesting hypotheses of relationships, the most significant result being recognition of zosterophyllopsids, lycopsids, and of several basal lineages not yet well known. These analyses increase our understanding of lycophytes, zosterophyllopsids and lycopsids as natural groups, but do not at this stage help significantly to clarify relationships within and between these groups. Moreover, some characters and possibly character states employed in these phylogenetic studies require modification or re-interpretation in order to more fully resolve relationships. For example, the phylogenetic positions of several plants more traditionally viewed as zosterophylls remain unresolved as do the relationships of presumed basal lycophytes from China which show extremely interesting combinations of characters. Interpretation and understanding of important morphological characters and character-state transformations which appear to be critical in delineation of major clades are presently particularly unsatisfactory, and remain a major obstacle to resolution of early lycophyte phylogeny. Important examples include helical versus linear insertion of sporangia, presence/absence of circinate vernation, and inter-

pretation of sporangial shape, orientation, and mode of dehiscence. Re-evaluation is also particularly needed in linking early lycopsids (?protolepidodendrids) to the bipolar, increasingly arborescent Late Devonian/Carboniferous forms. Although we can view a simple transformation sequence of, for example, the Middle Devonian protolepidodendralean fertile leaf via the Middle / Upper Devonian *Longostachys* sporophyll to the compacted Carboniferous cone, we do not see a corresponding pattern in the evolution of the stigmarian rooting system using the conventional homologies of rootlets and leaves. With dramatic advances in our knowledge of fossils as likely in the next decade as has occurred during the past three decades, and perhaps better understanding of the developmental basis for evolutionary change, we anticipate greater understanding of the early radiation of lycophytes in the years to come.

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Isoetalean Lycopsid Evolution: from the Devonian to the Present

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ABSTRACT.—The evolution of the isoetalean lycopsids spans much of the history of vascular plants, from Late, (or possibly Middle), Devonian to the current day genus *Isoetes*. The best known fossil members of this group are the arborescent lepidodendrids that dominated the Late Carboniferous coal swamps. Simpler unbranched isoetaleans with elongate stems also predated, coexisted with, and postdated the coal swamp trees, extending well into the Mesozoic. Whereas certain synapomorphies such as stigmarian rootlets, bipolar growth and secondary tissues unite the clade, other features characterize smaller subgroups of differing age, growth form and possibly, evolutionary lineage. Although some of these features are well known for plants of given time periods, particularly the Carboniferous, trends in character evolution have never been adequately documented for the group as a whole. A better understanding of such trends throughout the isoetalean fossil record could be valuable in distinguishing evolutionary lineages from convergence. It is interesting that several morphological characters of modern *Isoetes* are present as early as the Triassic: monolete microspores, sunken sporangia and elaborate ligules with glossopodia occur within elongate-stemmed Triassic forms. The dominant plant habit of modern *Isoetes*, a reduced cormose form that lacks appreciable stem elongation, originated at least by the Jurassic and typifies late Mesozoic and Cenozoic isoetaleans.

The lycopsids have always held a particular fascination for paleobotanists and neontologists alike because of their diverse morphologies, long stratigraphic record and their phylogenetic position as the outgroup to the rest of the vascular plants (Gifford and Foster, 1989; Kenrick and Crane, 1997). Whereas most lycopsids are similar to other pteridophytes in their growth habit and life history, isoetalean plants are characterized by distinctive morphological innovations, including secondarily derived bipolar growth, secondary cortical (and possibly vascular) tissues and arborescence. This group is generally thought to make its first appearance in the Late Devonian, or perhaps earlier, and reach its greatest diversity and ecological importance in the Late Carboniferous. Isoetaleans continue into the Early Mesozoic, where they are represented worldwide by numerous species of *Pleuromeia* Corda, *Annalepis* Fliche and several other forms. In the Jurassic, plants quite similar to modern *Isoetes* L. first appear. They continue to diversify throughout the Cretaceous and Tertiary and up to the present day.

The best known members of the Isoetales are the arborescent lepidodendrids, a group that dominated the Late Carboniferous (= Middle-Upper Pennsylvanian) coal swamp of Euramerica. These heterosporous trees had multibranched crowns of varying forms, long microphyllous leaves attached to characteristic diamond- or hexagonal-shaped leaf cushions, thick secondary cortical tissues, well developed stigmarian rooting systems and often monosporangiate cones with reduced numbers of megaspores and specialized

modifications for dispersal (Phillips, 1979; Bateman *et al.*, 1992; Phillips and DiMichele, 1992). Most of the arborescent forms became extinct by the Late Carboniferous of Euramerica concomitant with the loss of the most extensive coal swamp habitats (Bateman, *et al.*, 1992; Stewart and Rothwell, 1993). However, growing alongside the Middle Pennsylvanian tree lycopsids and surviving after them into the Late Carboniferous (Upper Pennsylvanian) were smaller (1–2 m tall), unbranched or rarely branched isoetaleans such as *Chaloneria* Pigg & Rothwell and *Sporangiostrobus* Bode (Pigg and Rothwell, 1979, 1983a, 1983b, 1985; Wagner, 1989). It is now known that isoetaleans with this elongate, unbranched growth habit originated by the Late Devonian (e.g., *Clevelandodendron* Chitaley & Pigg, Chitaley and Pigg, 1996) and were widespread throughout the Triassic where they belonged to at least two broad groups, *Pleuromeia*-like and *Annalepis*-like forms and their relatives (Pigg, 1992; Grauvogel-Stamm and Lugardon, 2001). Today the order Isoetales is represented by a single genus, *Isoetes,* which remains one of the most unusual living pteridophytes and retains many morphological, anatomical and developmental features that reflect its origins (e.g., Stewart, 1947; Gifford and Foster, 1989; Bateman, *et al.*, 1992; Pigg, 1992).

Recent studies have provided new information about the origin, evolution and diversity of the Isoetales. In this contribution, four phases of evolution in isoetalean lycopsids are addressed: (1) presumed Devonian origins of the Isoetales; (2) their maximum diversity in the Late Carboniferous, (3) Mesozoic plants with the growth habit of modern *Isoetes,* (4) new occurrences of Cretaceous and Tertiary forms. Lastly, problems of relating fossil taxa to extant *Isoetes* are addressed and suggestions are outlined for a better understanding of the isoetalean lineage through time.

Origin of Isoetalean Lycopsids

Historically, the Isoetales, the rhizomorphic heterosporous lycopsids, have been assigned to a variety of orders including the Lepidodendrales, Pleuromeiales, Isoetales and some forms occasionally to Protolepidodendrales (Chaloner, 1967; Pigg and Rothwell,1983b; DiMichele and Bateman, 1996; Kenrick and Crane, 1997), and even, probably mistakenly, the Selaginellales (see Rothwell and Erwin, 1985). In this paper I follow DiMichele and Bateman (1996), who recognize the entire group within the order Isoetales, based on a suite of synapomorphies including bipolar growth, secondary tissue production, and stigmarian rootlet formation.

Whereas much is known about both vegetative and reproductive structure of fossil isoetaleans, particular attention has been given to the basal parts, or rhizomorphs of the plants, especially the size and shape of their lateral lobes or horns. A great deal of diversity has been documented for these rhizomorphic features which vary from elongate, dichotomizing rooting systems of *Stigmaria* Brongniart to smaller bilaterally symmetrical lobed or rounded forms (e.g., *Pleuromeia*), to radial unlobed forms (e.g., *Paurodendron* Fry). All of these rhizomorphic forms can be homologized (Rothwell and Erwin, 1985; Grauvo-

gel-Stamm and Lugardon, 2001). It is now clear that the shape of the rhizomorph, or even its symmetry, is not nearly as significant as its production of stigmarian appendages or "rootlets" with their characteristic anatomy and the likelihood of their secondary derivation from an aboveground shoot system. This fascination with the basal portions of the plants has been particularly centered around the stigmarian rooting system and its ubiquity among Late Carboniferous coal swamp lepidodendrids. There has been interest for some time in finding evidence for the origin of *Stigmaria*, with the underlying assumption that this would lead toward understanding the origin of the isoetalean clade as a whole. Devonian and Early Carboniferous plants that obtained tree stature and/or those which produced rhizomorphic plant bases have been sought as the ancestors of lepidodendrids. The best known candidates have been the enigmatic "Naples tree" *Lepidosigillaria whitei* Kräusel & Weyland from New York State and *Cyclostigma kiltorkense* Haughton, from Late Devonian strata in several areas of Ireland and Bear Island in the Arctic, and China.

Lepidosigillaria whitei Kräusel & Weyland, a large stem with a plant base that appears rounded and lacking a stigmarian rooting system, occurs in the Late Devonian of New York State (Grierson and Banks, 1963). The original specimen was over 5 m tall and demonstrated that well developed, arborescent lycopsids had clearly evolved by the Late Devonian (White, 1907; Kräusel and Weyland, 1949; Grierson and Banks, 1963; Chaloner, 1967). Because of lateral compression during fossilization, the exact nature of the plant base of *Lepidosigillaria* is unknown. However, there is some increased thickening of basal cortical tissues, suggesting secondary tissues were produced. Several distinctive decortication layers are recognized on the stem surface, and small (less than 3 cm long), very narrowly attached leaves occur at higher levels. Although *Lepidosigillaria* has been described as having rootlets attached to the plant base (White, 1907), preservation is poor and their presence cannot be verified (Gensel and Berry, 2001).

A second Late Devonian lycopsid, *Cyclostigma kiltorkense* Haughton, has also been difficult to interpret. This plant was originally described from the Upper Devonian of Ireland, and later found in Bear Island, in the Arctic, and China (Johnson, 1913; Chaloner, 1967, 1968; Schweitzer, 1969; Cai and Wu, 1994). *Cyclostigma* is described as a large, heterosporous plant with characteristic rounded leaf scars (Chaloner, 1967) and is reported to have a bilobed plant base (Johnson, 1913). Specimens showing plant bases may be known (Chaloner, pers. comm. 1999) but they have never been clearly illustrated and have yet to be studied in detail. It also remains unclear whether all plant remains assigned to this genus represent the same plants. Clearly, reinvestigation of this problematic genus is in order.

Despite the currently limited information about *Lepidosigillaria* and *Cyclostigma*, their occurrences have established the presence of large isoetalean plants at least as early as the Late Devonian. In recent years additional Late Devonian and older, Middle Devonian lycopsids with a presumed relationship to the Isoetales have been reported. The Middle Devonian *Longostachys latis-*

FIG. 1. Line diagram of the rootstock of *Longostachys latisporophyllus*. Redrawn from Cai, Chongyang, and Lizhu Chen, 1996.

porophyllus Zhu, Hu & Feng *emend.* Cai & Chen was described from Givetian strata of southern China (Cai and Chen, 1996; Gensel and Berry, 2001). *Longostachys* is reconstructed as a small, branching heterosporous lycopsid tree that was up to 1.5 m tall with a stem 3.5 cm wide and a plant base that had a three-dimensional funnel-like structure with thick, rootlike appendages (Fig. 1). Because of its bipolar growth and centralized rooting system, this Middle Devonian plant may have some significance to isoetalean origins. The rooting structure of *Longostachys* bears some resemblance to stigmarian appendages, however it apparently lacks the dichotomizing stigmarian axes and characteristic stigmarian rootlet scars. Given the plant's Middle Devonian age it may be that these rooting organs are modified branching axes that cannot be clearly defined as stem or root, and may be more comparable to the H & K branching characteristic of zosterophylls (Gensel and Berry, 2001). However, well-defined leaves and strobili are produced by *Longostachys,* indicating that true leaves and stems had evolved by then. Whatever their exact homologies, the rooting structures of *Longostachys* suggest a potential intermediate step in the evolution of bipolar growth and the origin of stigmarian rooting systems in isoetaleans.

Additional Late Devonian isoetaleans have been discovered in Pennsylvania and Ohio. A flora from Red Hill, Pennsylvania contains cormose lycopsid plant bases (Cressler, 1998, 1999; Scheckler, *et al.*, 1999). Five specimens with attached rootlets have been recovered from oxbow lake facies of this fluvial deposit. One specimen has a stem width of 10 cm and a flared base of 12 cm, and with two lobes visible and is estimated to have four lobes. A second specimen is approximately 2 cm wide with a flared base. Roots are borne in orthostichies, with largest roots adjacent to one another. Associated, usually un-

branched, stems have various decortication patterns including *Knorria* Sternberg, *Helenia* Zalessky and *Cyclostigma*, as well as *Lepidodendropsis* Lutz-like pseudowhorls. Reproductive structures are not yet known (Walter L. Cressler, University of Pennsylvania, pers. comm. 1999). The Elkins, West Virginia locality that provided the earliest seed plant, *Elkinsia*, is of comparable age to Red Hill, and also contains lycopsid stems but plant bases are not yet known from Elkins (Gar W. Rothwell, Ohio University, pers. comm.1999).

Another recently recognized Late Devonian plant with an elongate isoetalean plant habit is *Clevelandodendron ohioensis* Chitaley & Pigg from the Cleveland Shale (Chitaley and Pigg, 1996). The genus is based on a single specimen which represents the entire plant. *Clevelandodendron* is an unbranched, slender plant with a compact terminal bisporangiate cone at the apical end and a partially preserved plant base proximally. The specimen is 125 cm long from base to apex and 2 cm wide for most of its length. Although details of the plant base are obscure, it appears to bear several thick appendages that taper distally and an elongate stigmarian root system is lacking. The terminal bisporangiate cone contains trilete megaspores that are laevigate (smooth) and trilete microspores that conform to either *Calamospora* Schopf, Wilson & Bentall or *Punctatisporites* (Ibrahim) Potonié & Krempf. Ligules are not known. *Clevelandodendron* is of particular significance because it demonstrates that elongate, slender isoetalean lycopsids with an unbranched plant habit, more common in later isoetalean forms, were present as early as the Late Devonian.

Plant remains with similarities to *Clevelandodendron* are also known in younger, Lower Carboniferous strata at several localities in eastern North America, France and Ireland. For example, anatomically preserved stems of similar dimensions are present in Lower Carboniferous localities in France (Brigitte Meyer-Berthaud, Université Montpellier, pers. comm. 1996) and additional permineralized stems showing cortical and stelar features transitional between the traditional Protolepidodendrales and Lepidodendrales occur at a variety of localities in North America, (e.g., Andrews, Read and Mamay, 1971; Cichan and Beck, 1987; Roy and Matten, 1989), Ireland (Matten, 1989; Roy and Matten, 1989), Scotland (Beck, 1958), and France (Meyer-Berthaud, 1984). Several bisporangiate cones with spore types similar to those of *Clevelandodendron* have been described from Upper Devonian and Lower Carboniferous strata of France and eastern North America (Arnold, 1933, 1935, 1939; Mathews, 1940; Meyer-Berthaud, 1984). These bisporangiate cones with laevigate (smooth) megaspores are unlike the stratigraphically younger bisporangiate cones of *Flemingites* Carruthers. The genus *Flemingites* bears *Lycospora* Schopf, Wilson & Bentall microspores with an equatorial flange, and *Lageniculа* (Bennie & Kidston) Potonié & Krempf or *Lageniosporites* Potonié & Krempf megaspores with an apical spore extension called a gula (Brack-Hanes and Thomas, 1983). Thus, not all Paleozoic bisporangiate lycopsid cones can be assigned to *Flemingites* (Chitaley and Pigg, 1996). Species of *Flemingites* are thought to have been borne by small lepidodendrid trees such as *Paralycopodites brevifolius* (Williamson) DiMichele (e.g., Brack, 1970; DiMichele,

1980). Thus there may be at least two lineages of Paleozoic lycopsids that produced bisporangiate cones: those related to *Clevelandodendron* and those bearing *Flemingites* cones.

One interesting Lower Carboniferous plant of isoetalean affinities is the *Protostigmaria/Lepidodendropsis* plant. *Protostigmaria* Jennings plant bases are reported to have had up to 13 lobes and a maximum width of 32 cm. This multilobed rooting structure was initially described by Jennings (1975) and later studied by Jennings, Karrfalt and Rothwell (1983). These authors suggested that *Protostigmaria* grew like *Isoetes,* with new lobes being produced by forking at the ends of furrows. The presumed above-ground parts of *Protostigmaria,* assignable to *Lepidodendropsis* Lutz are found both at the same and at several additional nearby sites in the Mississippian Price Formation of Virginia (Patricia G. Gensel, personal communication, 1999). Above-ground parts of *Lepidodendropsis* suggest the plant was a tree that branched dichotomously and may have had determinate growth. It was apparently eligulate but heterosporous and had linear leaves. Middle Devonian fossils assigned to *Lepidodendropsis* may represent a mix of several different plants that may not be equivalent to this Lower Carboniferous plant. Study and correlation of *Protostigmaria* plant bases and vegetative stems at several decortication layers of *Lepidodendropis* and reproductive remains from the same localities will provide the opportunity to reconstruct this interesting Lower Carboniferous lycopsid (Gensel and Pigg, in progress).

Late Carboniferous Diversity

During the Late Carboniferous, diversity and ecological importance of isoetalean plants reached their maximum. Arborescent lepidodendrids are extensively well known as both permineralizations in coal ball floras, and as coalified compressions. Whole plant reconstructions, reproductive biology and paleoecological aspects of these plants have been reviewed elsewhere (e.g., Phillips, 1979; Bateman, et al., 1992).

The extensive information available about Carboniferous lepidodendrids has allowed for the whole plant reconstruction of several species including the bisporangiate *Paralycopodites,* and larger monosporangiate trees with specialized dispersal units that have been modified presumably for water dispersal (Phillips, 1979). These include *Diaphorodendron* DiMichele and *Synchysidendron* DiMichele & Bateman, genera with two different crown branching forms that both produce *Achlamydocarpon varius* (Baxter) Taylor & Brack-Hanes, a megasporangium with a specialized, thickened wall surrounding a single functional megaspore (DiMichele and Bateman, 1992). Another tree lycopsid, *Lepidophloios* Sternberg, produces *Lepidocarpon* Scott, a cone that breaks up into individual megasporophyll units that contain the megasporangium with a single functional megaspore surrounded by an elongate and elaborated sporophyll (Phillips and DiMichele, 1992). Another tree form, *Sigillaria* Brongniart, bore monosporangiate cones on lateral cauline peduncles. Permineralized megasporangiate cones of *Sigillaria, Mazocarpon* (Scott) Benson produced 4–8 large

megaspores that are found each associated with a portion of sporangial wall and a pad of sterile tissue (Schopf, 1941; Pigg, 1983; Phillips and DiMichele, 1992). Parallels of these specialized forms with the seed habit have been commonly made (e.g., Phillips, 1979; Thomas, 1981).

We now know that in addition to the large arborescent lepidodendrids, which themselves were quite diverse, isoetaleans had a variety of other plant habits. These included the small scrambling "pseudoherbs" *Oxroadia* Alvin and *Paurodendron* Fry, as well as the unbranched monopodial plants *Chaloneria* Pigg & Rothwell and *Sporangiostrobus* Bode. *Oxroadia,* a plant of Lower Carboniferous age, is known from a number of localities in England and Scotland (Bateman, 1992), while *Paurodendron* has been described from Middle and Late Pennsylvanian coal ball floras of eastern and midcontinent North America (Rothwell and Erwin, 1985). Comparative developmental studies of apical and lateral growth of rhizomorphs in *Paurodendron, Stigmaria, Nathorstiana* Richter and modern *Isoetes* have been instrumental in understanding homologies across the isoetalean clade (Karrfalt, 1984; Rothwell, 1984; Rothwell and Erwin, 1985; Rothwell and Pryor, 1991).

The genus *Chaloneria* is reconstructed as a whole plant based on permineralized stems, fertile areas with *in situ* spores, decortication surfaces, and megagametophytes (Pigg and Rothwell, 1979, 1983a, 1983b, 1985). In contrast to the well known lepidodendrids, *Chaloneria* was more like its Devonian relative, *Clevelandodendron,* in having an unbranched, elongate stem. Instead of compact cones *Chaloneria* exhibits a less well differentiated fertile area that occurs either apically with alternating mega- and microsporangiate zones (*C. cormosa* Pigg & Rothwell) or alternating vegetative and fertile zones (*C. periodica* Pigg & Rothwell; DiMichele, Mahaffy and Phillips, 1979; Pigg and Rothwell, 1983a, 1983b). These and presumably related forms share the megaspore type *Valvisisporites* Ibrahim (Gastaldo, 1981) and microspores of *Endosporites* Wilson & Coe (e.g., Brack and Taylor, 1972; Hanes, 1975).

Compressed lycopsid reproductive structures bearing the same spore types as *Chaloneria* are given the name *Polysporia* Newberry (Chaloner, 1958; Drābek, 1976a, 1976b; DiMichele, *et al.* 1979; Pigg and Rothwell, 1983b). Previous reports of *Polysporia* were from incompletely preserved specimens, and it was difficult to tell whether these structures were compact cones or fertile areas such as those of *Chaloneria.* A recent study of well-preserved, complete cones of a new species of *Polysporia* from France, *P. doubingeri* Grauvogel-Stamm & Langiaux shows that *Polysporia* was at least sometimes a compact cone rather than an undifferentiated fertile area (Grauvogel-Stamm and Langiaux, 1995). This study brings to question whether all plants that produced these characteristic spore types, well known from Middle Pennsylvanian coals (Pigg, 1992), had the same type of growth habit (Pigg and Rothwell, 1983b, Grauvogel-Stamm and Langiaux, 1995).

Sporangiostrobus Bode is known from coal ball floras of the Middle Pennsylanian of Kansas and compressed floras from the Stephanian (= Upper Pennsylvanian) of Spain (Pigg and Rothwell, 1983b; Wagner, 1989 and references cited therein). This plant parallels the *Chaloneria/Polysporia* plants in

stem anatomy, size, unbranched to rarely branched habit and presence of fertile areas rather than compact cones. These plants grew in nearly monotypic stands, a situation also common in later pleuromeian forms (e.g., Retallack, 1975; Fuchs *et al.*, 1991; Pigg, personal observation, 1991). In contrast to *Chaloneria/Polysporia* plants, *Sporangiostrobus* bore the highly variable *Densosporites* (Loose) Schopf, Wilson & Bentall microspores, characterized by a prominent equatorial ridge, the cingulum, and *Zonalesporites* (Bartlett) Leisman megaspores. Whereas Pigg and Rothwell (1983b) suggested that *Sporangiostrobus* may be assignable to the same family as *Chaloneria,* the Chaloneriaceae, similarities of the two groups may be more the result of convergence in growth habit. Several other Carboniferous forms which, for various reasons, have been allied with the isoetaleans are reviewed by Pigg (1992).

Origin of modern *Isoetes* plant habit and morphological features

As the Paleozoic ended and most of the large, specialized lepidodendrid plants died out, a variety of several smaller, elongate, unbranched forms remained. These are represented in the Mesozoic by the genera *Pleuromeia* Corda, *Annalepis* Fliche and several other diverse forms (Pigg, 1992; Skog and Hill, 1992; Retallack, 1997; Grauvogel-Stamm and Lugardon, 2001). Around a dozen species of *Pleuromeia* have been named from numerous localities throughout the world including Germany, Russia, China, Japan and Australia (Pigg, 1992). Some pleuromeian genera, such as *Cylomeia* (White, 1981) and *Lycomeia* (Dobruskina, 1985) have been proposed on basis of the geographic occurrence, morphology and mono- *vs* bisporangiate nature of plants. However, whereas numerous species of *Pleuromeia* have been described and some are well known (e.g., Grauvogel-Stamm, 1993), the overall diversity and interrelationships of these taxa remain obscure.

In addition to pleuromeians there are a suite of Triassic plants assigned to *Annalepis* and related taxa which have been described by Grauvogel-Stamm and colleagues (Grauvogel-Stamm and Duringer, 1983; Grauvogel-Stamm and Lugardon, 2001). This latter group appears distinct from pleuromeians on the basis of a number of features including sporophyll morphology and spore characters. In some features *Annalepis* may resemble some later isoetaleans of Cretaceous-Tertiary ages (Grauvogel-Stamm and Lugardon, 2001).

A third source of Triassic lycopsids is the discovery of several permineralized forms. These include *Takhtajanodoxa* Snigirevskaya from Russia (Snigirevskaya, 1980a, 1980b), forms attributed to *Pleuromeia,* also from Russia (Snigirevskaya and Srebrodelskaya, 1986) and the more recently reported permineralized lycopsids from the Lower Triassic of Australia (Cantrill and Webb, 1998). The affinities of permineralized Triassic forms and their relationships to the more widely known compressed remains have not been fully resolved.

In the early 1980's Snigirevskaya described the permineralized Triassic plant *Takhtajanodoxa* from Russia (Snigirevskaya 1980a, 1980b). This plant has stem, leaf and megaspore anatomy similar to *Chaloneria* and other Pennsylvanian lycopsids. What is surprising, however, is the presence of elaborately

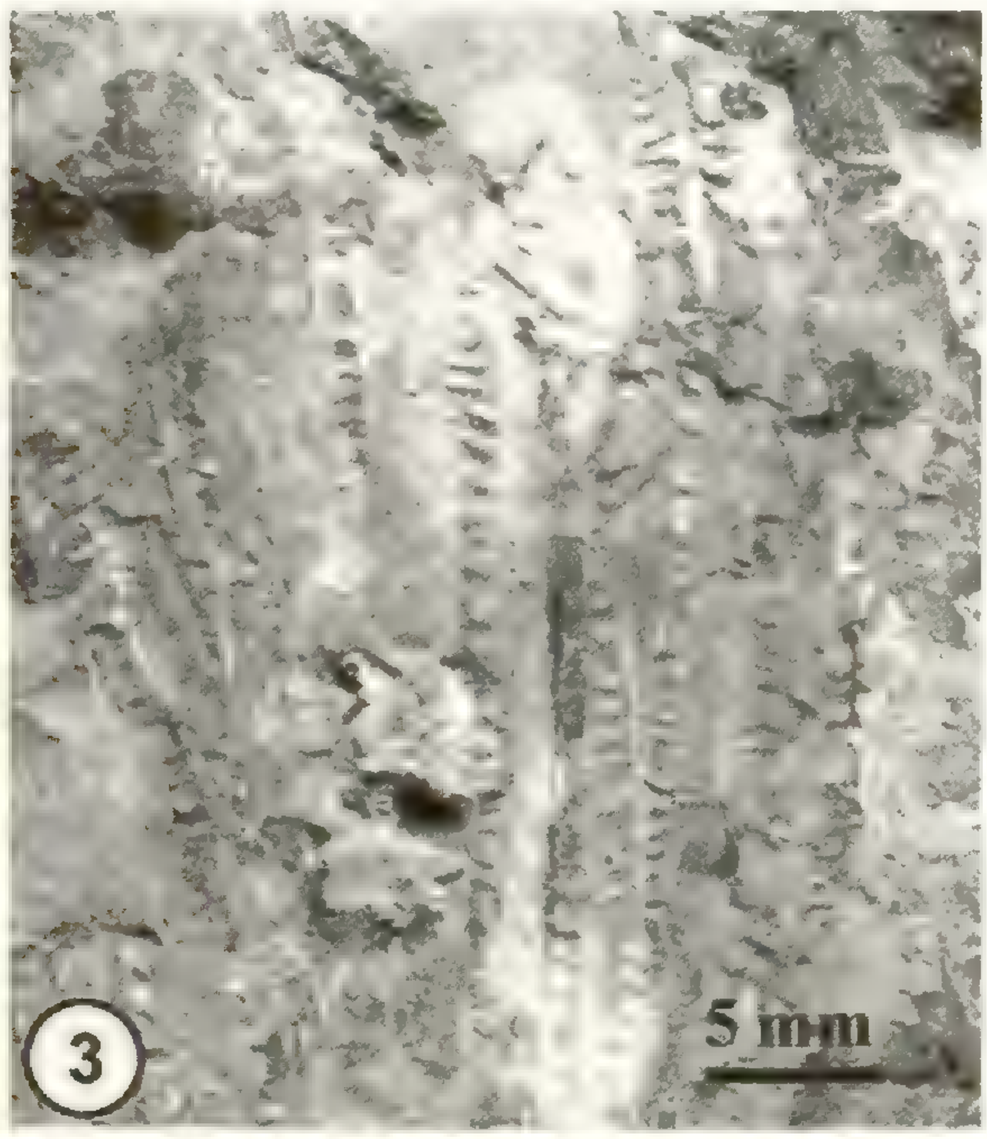
shaped ligules with curving basal extensions of the glossopodium (Snigirevskaya, 1980a, 1980b; Pigg, 1992). This type of ligular structure is lacking in Paleozoic permineralized lycopsids but present in extant *Isoetes* (Gifford and Foster, 1989). Permineralized stems described as *Pleuromeia* were also characterized from Russian Lower Triassic strata (Snigirevskaya and Srebrodelskaya, 1986). These specimens show small, protostelic stems lacking secondary tissues with mesarch leaf traces, and fimbriate metaxylem tracheids with scalariform and reticulate wall thickening patterns. More recently, permineralized forms have been described from the Lower Triassic Bowen Basin of eastern Australia by Cantrill and Webb (1998). These authors describe a suite of disarticulated lycopsid plant organs that include one bisporangiate and one monosporangiate cone type, two types of stems, one with prominent aerenchyma in the cortex, and one type of rooting structure that bears stigmarian-like rootlets. From the presently known information, at least two types of lycopsids thus appear to be present in these remains. As with late Carboniferous coal ball lycopsids, for the first time the exciting potential exists for "whole-plant" reconstructions of Lower Triassic isoetaleans, based on the co-occurrence, anatomical similarity and organic interconnections between these separate plant organs.

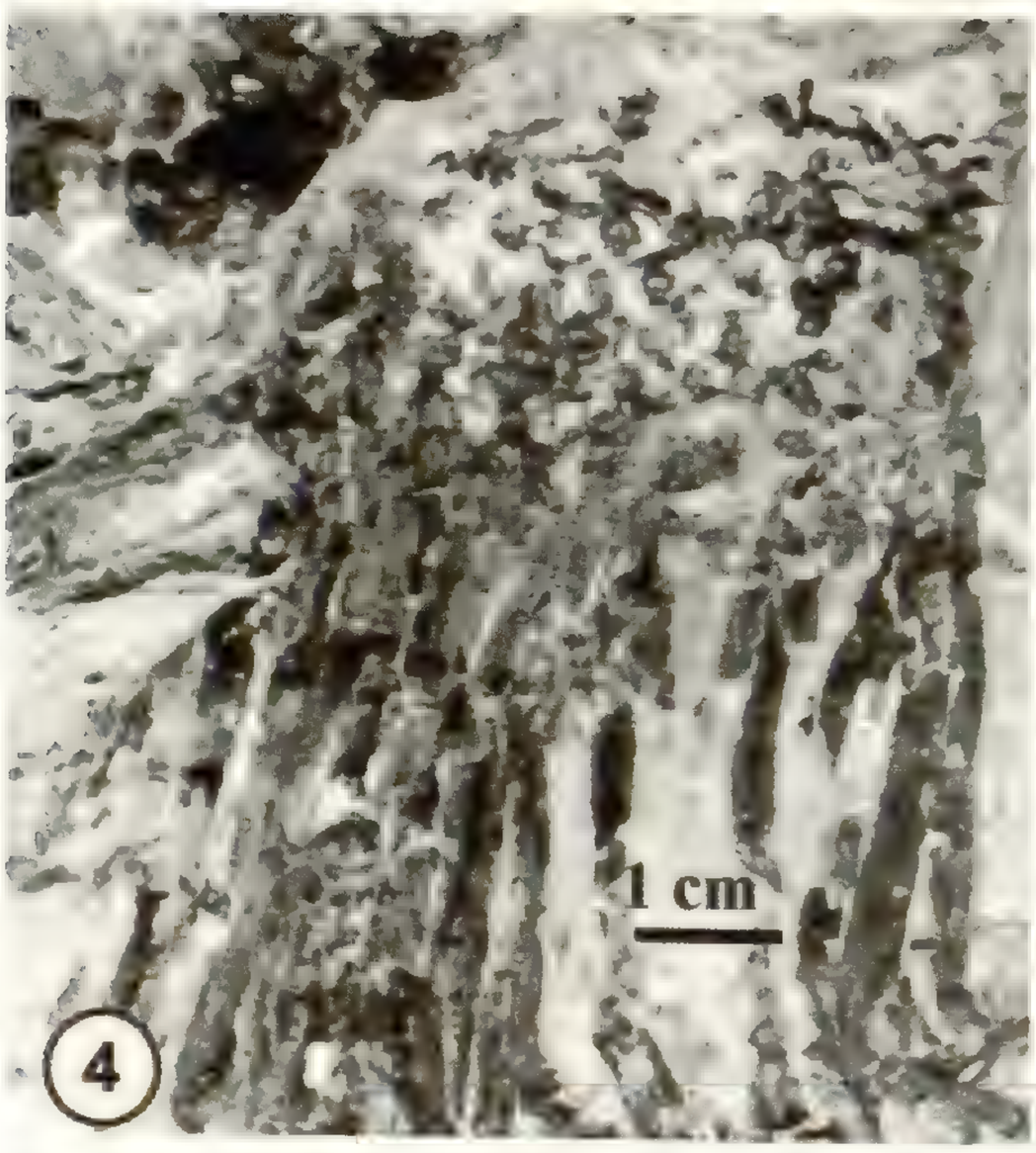
It is during the Mesozoic that we see the origin of a number of morphological features present in modern-day *Isoetes*. These features include: 1) a change in microspores from trilete to monolete suture (and probably also change in tetrad arrangement); 2) sporangia that are sunken into the adaxial surface of the sporophyll; 3) an elaboration of the basal portion of the ligule into a glossopodium; 4) the origin of a velum and/or a labium, covering the proximal, distal, or entire upper surface of a sporangium.

All of these features can be seen among various Triassic-aged plants. Microsporophylls of *Annalepis* are characterized by monolete microspores of the *Aratrisporites* (Leschik) Playford & Dettman type (Grauvogel-Stamm and Lugardon, 2001). Sporangia are notably sunken into the sporophyll surface in several species of *Pleuromeia* such as *P. rossica* Neuburg and *P. longicaulis* (Burges) Retallack (Neuburg, 1960, 1961; Retallack, 1975). Ligules with an extended basal glossopodium much like that present in many extant species of *Isoetes* occur in the permineralized Triassic Russian plant *Takhtajanodoxa* (Snigirevskaya 1980a, 1980b; Pigg, 1992).

Whereas it appears that no single currently known Triassic isoetalean taxon possesses all of these features, all are found within various representatives of Triassic lycopsids. As the Triassic and later Cenozoic lycopsids are better documented, it will be possible to better understand character evolution in the various lineages of Triassic lycopsids (Grauvogel-Stamm and Lugardon, 2001). This suggests the Triassic was a time of important radiation and change in several key morphological characters prior to the appearance of the extant, reduced *Isoetes* forms.

The origin of the modern *Isoetes* plant habit with a nonelongated stem was previously thought to occur in the Triassic (Pigg, 1992). However, evidence for this type of plant habit is scant, as the best documented Triassic *Isoetes*-like





FIGS. 2–5. Tertiary and extant isoetalean lycopsids. Fig. 2. cf. *Isoetites* from Dennison Gap, Wyoming. Florida Museum of Natural History. UF 18120–12604. Fig. 3. Detail of specimen from Dennison Gap, showing square air channel pattern on sporophyll surfaces. Florida Museum of Natural History. UF 18120–12604. Fig. 4. Joffre Bridge isoetalean plant. University of Alberta 539–421. Fig. 5. *Isoetes tegetiformans* habit shot.

plant, *I. gramineoides* (Ward) Bock, originally considered a grass, has been reidentified as a fern petiole (Bock, 1962; Skog and Hill, 1992). Another well known form, *I. circularis* from the Triassic of eastern North America, more closely resembles the genus *Annalepis* in sporophyll features, and its plant corms, whether elongate or reduced, have not been well documented (Grauvogel-Stamm and Lugardon, 2001). Recently, several authors have proposed additional *Isoetes* species in the Triassic (e.g., Wang, 1991; Retallack, 1997).

Although it is clear that all of these forms are isoetalean plants, none show undisputed evidence of a non-elongating stem such as is usually characteristic of modern *Isoetes*. They should certainly be included within the Isoetales, based on their particular morphological features, yet I am reluctant to accept them as belonging to the modern genus.

The earliest strong evidence of the modern plant habit is that seen in the Jurassic *Isoetites rolandii* Ash & Pigg from western North America (Ash and Pigg, 1991). Concomitant with this change in plant habit is the loss of vegetative leaves: modern *Isoetes* and its younger fossil relatives typically produce only fertile leaves, thereby minimizing the vegetative phase of its life history.

Cretaceous and Tertiary—Some New Occurrences

Over a dozen *Isoetes*-like plants have been described in Cretaceous and Tertiary strata (see Pigg, 1992, and Skog and Hill, 1992 for review). Several newly recognized forms have been recently described. One unusual example is *Monilitheca* Krassilov & Makulbekov described from the Upper Cretaceous of Mongolia by Krassilov and Makulbekov (1996). The genus consists of fragmentary linear megasporophylls up to 18 mm long and 1 mm wide that bear within the sporangium, a single row of megaspore tetrads. These tetrads are borne along almost the entire length of the megasporophyll, which has a short sterile tip. Megaspores are trilete, and reticulate.

Isoetalean plants described from the Lower Cretaceous of Tunisia were named *Isoetites daharensis* Barale (1999). These fossils are represented by corms 10 cm long × 6 cm wide with attached sporophylls and roots. Sporophylls are ligulate and bear sporangia containing trilete megaspores 480 μm in diameter. Isoetalean plants have also been recognized in the lower Cretaceous of Brazil, in the Santana Formation (Dilcher *et al.*, 2000). In this flora, *Isoetes*-like plants are represented by clusters of sporophylls that occur along with ferns, conifers, angiosperms, and abundant gnetalean remains.

In the Tertiary, plants from the Paleocene Joffre Bridge locality in central Alberta, Canada originally identified by Hoffman as lilean monocots are clearly isoetalean (Hoffman, 1995, Plate 14, figs. 101, 102). The specimen is a plant base approximately 5.5 cm long and around 3 cm high with a crown of elongate leaves at the top of the specimen. At the base dense, helically arranged, rounded stigmarian rooting scars are present, a feature diagnostic for the isoetalean clade (Fig. 4). Around twenty stigmarian rootlets, each measuring 3 mm wide and over 7 cm long, are found in attachment to the plant base.

Additional isoetalean specimens are known from the Lower Eocene Dennison Gap locality of the Wasatch Formation near Sweetwater, Wyoming, where they occur with platycaryoid foliage, fructifications and *Azolla* (Steven R. Manchester, Florida Museum of Natural History, written communication, 1992; Fig. 2, 3). These specimens are probably assignable to either *I. horridus* or *I. serratus* of Brown, depending on details of the leaf margin that are not readily visible, and show the characteristic air channels that are often found in *Isoetites* leaves (Fig. 3; Brown 1939, 1958, 1962).

Conclusions and Suggestions for Further Research

In recent years, valuable new types of information have appeared that promise to help clarify long-held questions about the evolution of the isoetalean clade. Notable are newly discovered 1) Middle Devonian forms from China that may have some bearing on the origins of this order, 2) permineralized Triassic fossils that provide a missing "middle" to our knowledge of anatomical features between Paleozoic forms and extant *Isoetes*, and 3) Cretaceous and Tertiary forms that document additional diversity of the group. Phylogenetic analyses of anatomically preserved Carboniferous forms have been successful in delimiting interrelationships among the most complex isoetaleans which have been documented as "whole plants" and are understood ecologically (e.g., Bateman *et al.*, 1992; Phillips and DiMichele, 1992). However, to date it has not been possible to extend these types of studies beyond that stratigraphic range because of the limitations of preservation and difficulty of homologies.

Whereas researchers studying Devonian, Pennsylvanian or Triassic aged fossils or extant *Isoetes* have made partial comparisons, homologies of characters through geological time have been more difficult. Part of the problem has been the use of terminology developed for one group of lycopsids for a different group. In some cases, terms and concepts have had to be "stretched" to accommodate real differences between isoetalean lycopsids of different ages. What is needed is an analysis of overall diversity within given characters, such as sporophyll morphology, without terminology imposed *a priori*.

Lastly, with all of the work done on fossil and extant isoetaleans to date, the question of how to define the modern genus *Isoetes* in relation to its fossil relatives has never been resolved. In the present contribution I have referred to the extant *Isoetes* plant habit as having a reduced, non-elongating stem. However, there is considerable morphological variation in extant species. In addition to "typical" plants there are forms with branching axes such as *I. andicola* (formerly *Stylites* Amstutz), and the unusual rhizomatous mat-forming *I. tegetiformans* Rury (Fig. 5). Some species of *Isoetes* have even been observed to have small rhizomorphic structures (W. Carl Taylor, pers. comm. 1999).

However, despite this diversity, it is well known among neontologists that morphology has been of little value in understanding speciation in *Isoetes* because of the high degree of homoplasy and convergence to habitat (e.g., Taylor and Hickey, 1992). Despite numerous discussions, it is still unclear what is basal in the group based on morphology because of the strong imprint of ecological influence. Molecular studies that clarify extant species relationships may help us resolve this problem and offer clues to the relationships of the extant and relatively recent fossil isoetaleans. Further developmental studies of *Isoetes* may also aid in a better understanding of homologies. Nevertheless, the fossil record still promises to resolve much more about diversity and evolution within this interesting group throughout its long evolutionary history.

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The Triassic Lycopsids *Pleuromeia* and *Annalepis*: Relationships, Evolution, and Origin

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ABSTRACT.—Two kinds of isoetalean lycopsids widely prevailed in the Triassic, the *Pleuromeia*-type and the *Annalepis*-type, the latter including a plexus of closely related genera. Comparative studies using new macromorphological and ultrastructural data suggest that both genera are interconnected and closely related to *Isoetes*. Morever they suggest that *Annalepis* is probably ancestral to *Isoetes*, via *Isoetites*. Besides several of the morphological and ultrastructural features of the Triassic lycopsids and *Isoetes* also appear to be present in some of the most ancient lycopsids, suggesting that the lineage including the modern Isoetales has a very remote origin.

The lycopsids have the longest evolutionary history of any vascular land plants, covering about 400 million years and spanning every geological period from the Siluro-Devonian to the present. Although their structural diversity has decreased considerably since their peak in the Carboniferous, they were still widespread in the Triassic of both hemispheres. However the Mesozoic lycopsids were not arborescent like many of the Carboniferous ones but rather consisted of slender, herbaceous or pseudoherbaceous plants having an unbranched habit. Two kinds of lycopsids widely prevailed in the Triassic, the *Pleuromeia*-type and the *Annalepis*-type, the latter including a plexus of closely related genera, which are all regarded as belonging to the isoetalean lycopsids (Pigg, 1992).

Pleuromeia Corda was long regarded as one of the intermediates between the Carboniferous arborescent lepidodendrids and living *Isoetes* and thus thought to be part of a reduction series (Solms-Laubach, 1899; Potonié, 1904; Mägdefrau, 1931). This concept was questionned by Jennings (1975) who noticed that the lycopsids with a cormose rhizomorph already existed in the Upper Devonian, long before the lepidodendrids with a dichotomously branched *Stigmaria* rooting system. However DiMichele and Bateman (1996) also suggested, on cladistic foundations, that the isoetaleans evolved from the lepidodendraleans and that the bilateral condition emerged from the radial one in the Devonian. Nevertheless Bateman (1994, 1996) recognized that the precise relationships between both groups are ambiguous.

The comparative study of *Pleuromeia* and *Annalepis* presented here, using new macromorphological and ultrastructural data, shows that these Triassic isoetalean lycopsids appear closely related to *Isoetes* and that *Annalepis* seems to be closer than *Pleuromeia* to the living genus. Moreover this study consti-

tutes a new basis for comparison with other lycopsids and for evaluating and possibly clarifying the relationship between the isoetaleans and the lepidodendrids.

THE TRIASSIC LYCOPSID *PLEUROMEIA*: COMPARATIVE STUDY

Although the lycopsid *Pleuromeia* Corda was long regarded as being phylogenetically highly significant, it was never estimated at its true value because many characteristics of this widespread Triassic genus were not known with enough accuracy. The progress in knowlege of its rhizomorphs, growth habit, reproductive organs and spores now allows to propose a comprehensive comparative analysis of this outstanding genus.

THE RHIZOMORPH OF *PLEUROMEIA*.—As in the other rhizomorphic lycopsids (Bateman 1996), the rooting organ of *Pleuromeia* has proven to be, phylogenetically, highly informative since it shows many similarities with that of *Isoetes*, suggesting that these lycopsids are closely related. The reinvestigation and morphological analysis of several fairly well-preserved rhizomorphs of *P. sternbergii* (Münster) Corda in light of new data on *Isoetes* permit us to demonstrate the structural and developmental correspondence between both. Thus most of the features of the rhizomorph of *P. sternbergii* can be interpreted in terms of those of the living genus (Grauvogel-Stamm, 1993).

Like the rhizomorph of *Isoetes*, that of *Pleuromeia* is lobed and shows a bilaterally symmetrical furrow system, when seen from below (Figs.1a-f). The four-lobed rhizomorph of *P. sternbergii* has a central furrow bifurcating distally into two peripheral furrows which run along the midline of the lobes and extend upward into their extremities (Fig. 1c). Its roots have the same structure as those of *Isoetes* and *Stigmaria*. As in *Isoetes* but unlike *Stigmaria*, they are arranged on both sides of the furrows according to two intersecting row systems, one roughly parallel to the furrows (series) and the other diverging from them at a relatively high angle (orthostichies) (Fig. 1f). In the two-lobed rhizomorph of *P. epicharis* Wang and Wang (1990) from the Lower Triassic of China, the roots are also arranged in series and orthostichies on both sides of the straight furrow, as in the two-lobed rhizomorph of *Isoetes* (Figs.1a,b,e). This bilateral arrangement suggests that the roots were produced as in the living genus. That is, they are produced by a basal linear root-producing meristem underlying the lobed stele and running parallel to the furrow, and the roots emerged at the furrows and were progressively displaced by continued meristematic activity. The lobing of the rhizomorph developed in relation to this meristem and this process of root production. However the difference in cortical development in *Pleuromeia* and *Isoetes* resulted in a difference in lobe and furrow arrangement (Figs.1a-d). Indeed in *Isoetes* where the cortex is strongly developed, the peripheral furrows of a four-lobed rhizomorph seem to run between the lobes (Fig. 1d). In fact, the correct developmental correspondence requires comparing the lobes of *Pleuromeia* with those of the root-bearing stele in *Isoetes*, without its cortical lobes. Observations on the diameter

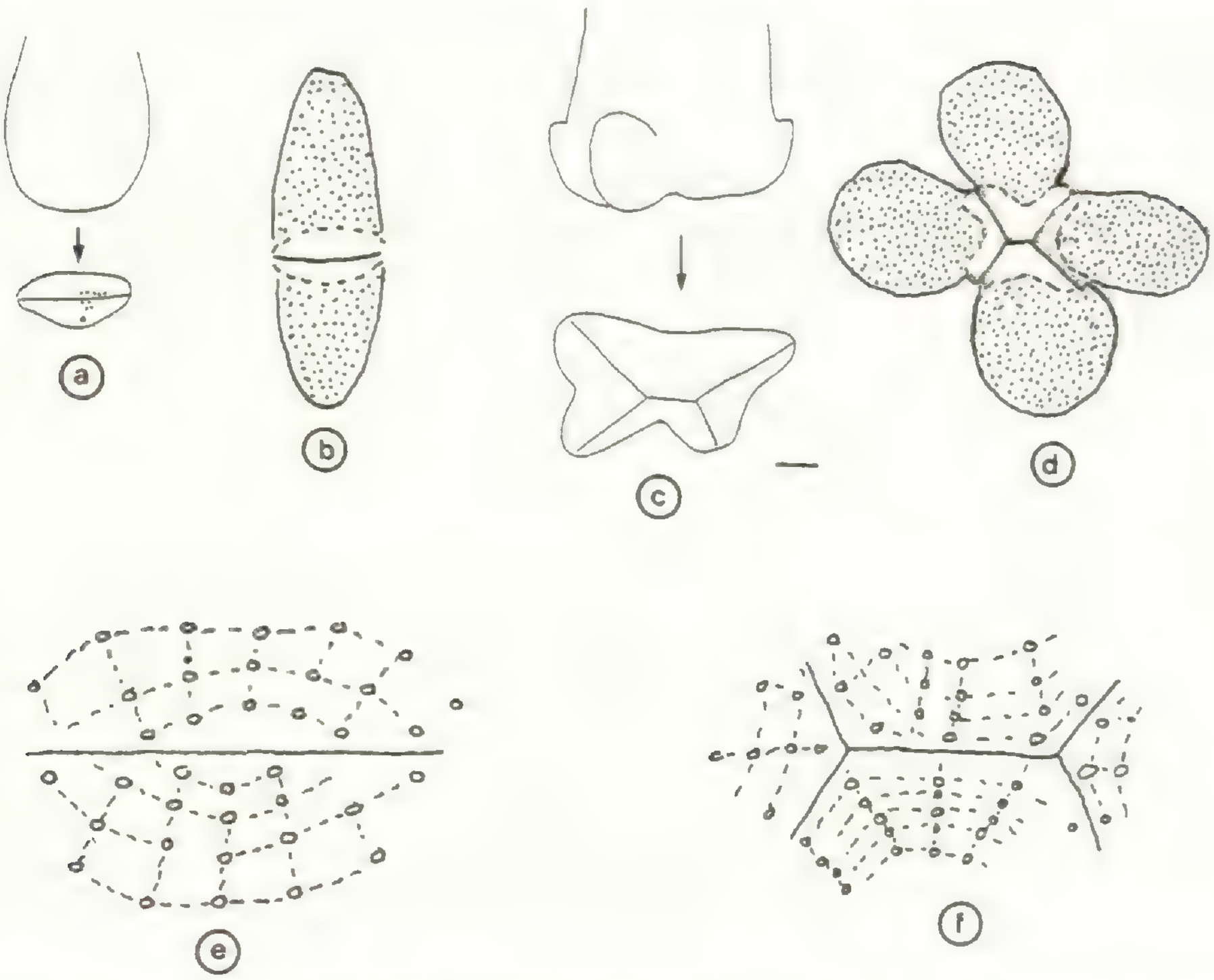

FIG. 1. Comparative morphology of the rhizomorphs of *Pleuromeia* and *Isoetes*. a—Side-view (above) and underside view (below) of a two-lobed rhizomorph of *Pleuromeia*, such as those of *P. epicharis* (Wang and Wang, 1990) or *P. sanxiaensis* (Meng, 1995) from the Triassic of China. b—Cross section of a two-lobed rhizomorph of *Isoetes* at the level of the basal meristem and furrow (inferred from Karrfalt and Eggert, 1977). This rhizomorph has a straight furrow like the two-lobed rhizomorph of *Pleuromeia* in Fig. 1a, but its two lobes (stippled) are more developed due to the great cortex production. c—Side-view (above) and underside view (below) of a four-lobed rhizomorph of *Pleuromeia*, like that of *P. sternbergii* (Grauvogel-Stamm, 1993) or *P. rossica* (Neuburg, 1960; Dobruskina, 1982). The bilaterally-symmetrical furrow system consists of a central furrow bifurcating at both extremities into two peripheral furrows which run along the midline of the lobes. d—Cross section of a four-lobed rhizomorph of *Isoetes* at the level of the meristem and furrow system (inferred from Karrfalt and Eggert, 1977). The furrow-system is similar to that of *Pleuromeia* in Fig. 1c, but the high cortex production resulted in the development of cortical lobes (stippled) between those of the root-bearing stele. Thus the correct developmental correspondence requires comparing the lobes of *Pleuromeia* with those of the root-bearing stele in *Isoetes* (cortical protrusions excluded). e—Root arrangement on both sides of the straight furrow in a two-lobed rhizomorph of *Pleuromeia* or *Isoetes*. The root scars are arranged according to two intersecting row systems, one roughly parallel to the furrow (series) and the other diverging at a relatively high angle (orthostichies) (modified from Karrfalt and Eggert, 1978). f—Root arrangement in series and orthostichies on both sides of the bifurcated furrow system in the four-lobed rhizomorphs of *Pleuromeia* or *Isoetes* (modified from Karrfalt and Eggert, 1978).

of the root scars of *P. sternbergii* in relation to their place on the rhizomorph demonstrate that root production proceeded in an acropetal direction as in *Isoetes*. The significant increase of the root scar diameter from youngest to oldest (also observed in *P. epicharis*, unpublished observations, L. G.-S.), their numerical increase with the aging and thickening of the rhizomorph, and the maintenance of their arrangement give evidence for long retention of the roots,

possibly their persistence throughout the life of the plant as in *Selaginella*. This is unlike *Isoetes* in which the roots are regularly lost and replaced. The sequence of root initiation, the orientation of the root traces inside the lobes and thus the direction of their emergence and the marked lengthening of the peripheral furrows which occurred during ontogeny show how the rhizomorph of *Pleuromeia* developed laterally to the detriment of its downward growth. This new restatement of the structure of the rhizomorph of *P. sternbergii* and the comparative study with the living *Isoetes* accurately emphasizes the numerous points of structural and developmental correspondence between these plants.

Fossil lycopsids with a cormose rhizomorph that is furrowed, lobed and bilaterally symmetrical are rather rare in the literature. Besides *P. sternbergii,* such a rhizomorph has been described in *P. rossica* Neuburg from the Lower Triassic of the Russian platform (Neuburg, 1960; Dobruskina, 1982) and *P. epicharis* from the Lower Triassic of China (Wand and Wang, 1990). The rhizomorphs of *P. sternbergii* and *P. rossica* are four-lobed and quite similar (Fig. 1c). In contrast, the rhizomorph of *P. epicharis* which is two-lobed, shows a straight, unbranched furrow (Fig. 1a). Also *P. sanxiaensis* Meng (1995) from the early Middle Triassic of South China has a two-lobed rhizomorph. Besides *Pleuromeia,* three other lycopsids with lobed, furrowed, and bilaterally symmetrical rhizomorphs have been described: *Protostigmaria eggertiana* Jennings from the Lower Carboniferous of Virginia, USA (Jennings, 1975; Jennings et al., 1983), *Nathostianella* Glaessner and Rao (1955) from the Lower Cretaceous of South Australia, and *Nathorstiana* Mägdefrau from the Lower Cretaceous of Germany (Karrfalt, 1984). Unlike *P. sternbergii,* they have no prominent lobes. In the lobed rooting organ of *Cylomeia* White (1981) from the early Triassic of Australia, no furrows have ever been mentioned and the other data are too imprecise to interpret this feature. In *Chaloneria cormosa* Pigg and Rothwell from the Pennsylvanian of North America, which is said to have a rounded, slightly lobed, and bilaterally symmetrical cormose rooting organ (Pigg and Rothwell, 1979; 1983; Pigg, 1992), the furrow system has not been mentioned and the root arrangement has not been described. The lobe and furrow system of *Chaloneria* was not fully documented because of the incomplete preservation of cortical tissue on plant bases of available specimens (Pigg, personnal communication, 1999). Among the cormose lycopsids, there are others in which the rhizomorph is radially or nearly radially symmetrical, such as *Paurodendron fraiponti* Fry from the Upper Pennsylvanian of Ohio, USA (Rothwell and Erwin, 1985) and *Oxroadia gracilis* Alvin from the Lower Carboniferous of Scotland (Stewart and Rothwell, 1993). Moreover, several other cormose lycopsids clearly lack an extensive branched rooting system but are not well enough preserved to permit a precise study of their rhizomorph. These are *Lepidosigillaria whitei* Kräusel and Weyland, *Cyclostigma kiltorkense* Haughton, possibly *Eospermatopteris erianus* (Dawson) Goldring from the Devonian (see Pigg, 1992, 2001), *Bodeodendron* Wagner/*Sporangiostrobus feistmantelii* (Feitmantel) Němejc from the Late Stephanian of Spain (Wagner, 1989) and *Clevelandodendron ohioensis* Chitaley and Pigg (1996) from the

Late Devonian of Ohio, USA. In all of the cormose lycopsids cited above, the structure of the rhizomorph, its development, and its root arrangement are not known with as much precision as in species of *Pleuromeia*.

The bilaterally symmetrical cormose rhizomorphs of *Pleuromeia* and *Isoetes* seem to differ greatly from the radially symmetrical stigmarian rooting organs of the lepidodendrids which are dichotomously branched and in which the roots are helically arranged. However, Rothwell and Erwin (1985) demonstrated that the radially and bilaterally symmetrical rhizomorphs are homologous and that they are merely growth variations rather than indicators of two major lines within the rhizomorphic lycopsids as suggested earlier by Jennings (1975) and Jennings et al. (1983). These homologies were the basis for including both the Isoetales and Lepidodendrales of the traditional classifications in the rhizomorphic or isoetalean clade (Rothwell and Erwin, 1985; Stewart and Rothwell, 1993; DiMichele and Bateman, 1996).

GROWTH HABIT AND REPRODUCTIVE ORGANS OF *PLEUROMEIA*.—The similarity in rhizomorphic structure which suggests a close relationship between *Pleuromeia* and *Isoetes* strongly contrasts with the differences in growth habit and reproductive structure of these plants. In *Pleuromeia* (Fig. 2c-g) the sterile leaves differ greatly from the fertile ones which form a well defined cone at the apex of the stem whereas in *Isoetes* (Fig. 2h, 7d), the fertile and sterile leaves are alike and do not form a cone (Jermy, 1990). However such differences seem to be frequent in related lycopsids. The genera *Polysporia* Newberry and *Chaloneria* which belong to the Chaloneriaceae and produce morphologically similar microspores and megaspores, are characterized by well-defined terminal cones in *Polysporia* (Grauvogel-Stamm and Langiaux, 1995) and by fertile zones in *Chaloneria* in which the sporophylls resemble the sterile leaves (Pigg and Rothwell, 1983). Likewise, in the extant genera *Phylloglossum* and *Huperzia* of the Lycopodiaceae which produce comparable spores, there is a compact strobilus in the first genus while the second genus has either fertile zones or compact strobili (Øllgaard, 1990).

All the *Pleuromeia* species, which have been discussed by Dobruskina (1985) and Pigg (1992), consist of unbranched plants having basal cormose rhizomorphs and terminal, well defined cones. The sizes of the plants are rather variable but many species are relatively small. Even among specimens of *P. sternbergii* from the type-locality (Bernburg, Germany) for which Mägdefrau (1931) indicated a height of 2–2.5 m, there are fertile specimens which only reach 23 cm (Fig. 2d) (Grauvogel-Stamm, 1999). Those from the Eifel in Germany (Fig. 2c) are 1–1.5 m tall (Mader, 1990; Fuchs et al., 1991). *Pleuromeia rossica* from the Lower Triassic of Russia was no more than 1 m high (Neuburg, 1960) and *P. jiaochengensis* Wang and Wang (1982) from the Lower Triassic of China usually reached 20–30 cm high, sometimes 50 cm (Fig. 2e). Recently, several new species have been described from the early Middle Triassic of southern China (Meng, 1995,1996) which consist of plants mostly 50 cm high. However, *P. marginulata* Meng (Fig. 2f) includes specimens more

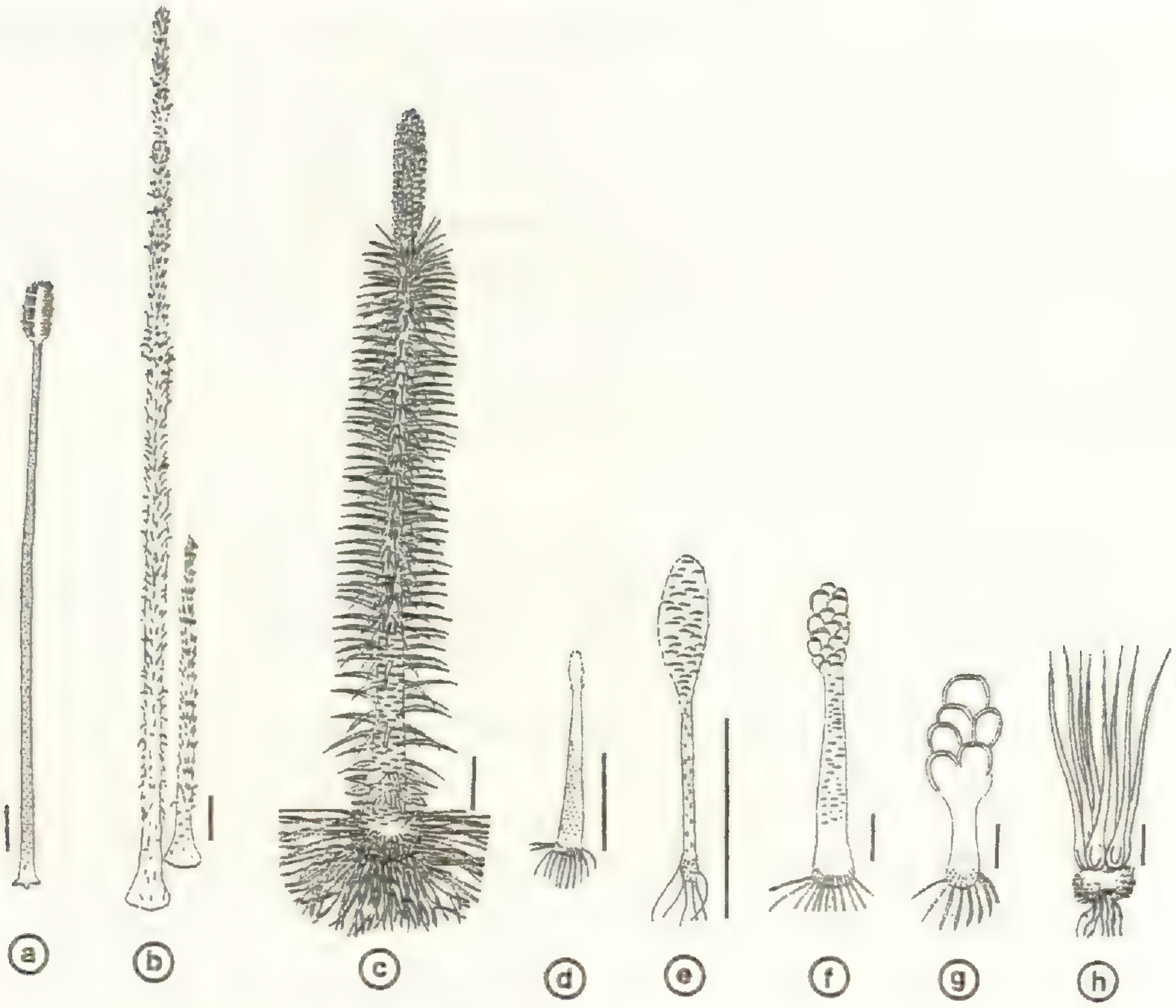

FIG. 2. Comparative growth habit of the Paleozoic and Triassic *Pleuromeia*-like lycopsids and the extant *Isoetes*. (scale-bar = 10 cm, except in Fig. 1g,h = 1 cm). a—*Clevelandodendron ohioensis;* Devonian of Ohio, USA (redrawn from Chitaley and Pigg, 1996). b—*Chaloneria cormosa;* Pennsylvanian of North America (redrawn from Pigg and Rothwell,1983). c—*Pleuromeia sternbergii;* Lower Triassic (Olenekian) of the Eifel, Germany (from Fuchs et al., 1991). d—*P. sternbergii* from the Lower Triassic of Bernburg, the type-locality, (reconstructed after the illustrations of Bischof, 1853, Figs.1,2). e—*P. jiaochengensis;* Lower Triassic (Induan) of Shanxi, China (redrawn from Wang and Wang, 1982). f—*P. marginulata;* early Middle Triassic (Anisian) of South China (reconstructed after the illustrations of Meng, 1995: Pl.1 fig.l and Meng, 1996: Pl.1 fig 1). g—*P. sanxiaensis;* early Middle Triassic (Anisian) of South China (reconstructed after the illustrations of Meng, 1995, 1996, Pl.1 Fig. 9). h—*Isoetes brochoni,* showing leaves 3–7 cm long (redrawn from Motelay, 1893).

than 1 m high. In contrast, *P. sanxiensis* Meng is only 5 cm tall, with a 2,5 cm long cone (Fig. 2g).

The trunk of *Pleuromeia* is usually devoid of leaves but is covered with helically arranged leaf scars. However some of the specimens of *P. sternbergii* from Eifel had trunks covered with leaves (Fig. 2c). These leaves have a strong midvein and, according to Mägdefrau (1931), they were succulent and contain transverse ridges extending perpendicularly on both sides of the midvein. As suggested by Grauvogel-Stamm (1999, Fig. 4), these transverse ridges might correspond to air chambers, as in *Isoetes* and *Isoetites phyllophilla* Skog, Dilcher and Potter (1992). However this feature requires further investigation.

Cone size is variable in *Pleuromeia*. According to Mägdefrau (1931), the cone of *P. sternbergii* reached 20 cm long. However, other cones of that species

are only 5 cm long (Bischof, 1853, Figs.1,2; Grauvogel-Stamm, 1999). Likewise according to Mägdefrau (1931), the cones were monosporangiate, containing either microspores or megaspores. Nevertheless, the small cone figured by Bischof (1853) is clearly bisporangiate (Grauvogel-Stamm and Lugardon, in preparation). Its microsporophylls and megasporophylls are inter-mixed as in *P. rossica* (Neuburg, 1960). The cones of *P. sternbergii* appear to be mono- or bisporangiate depending perhaps on the size and the age of the plants. The two cones on which Mägdefrau (1931) relied were rather large whereas that of Bischof (1853) is small. In *Isoetes* too, the size of the plants has an influence on the production of microsporophylls and/or megasporophylls (Williams, 1943).

The sporophylls of the different *Pleuromeia* species show a remarkable similarity in shape and structure since they consist of a circular to oval lamina and a large, globose sporangium which covers nearly all of the adaxial surface (Figs.3a-j). Distally, the margin has a slight notch or a tiny pointed tip at the apex. The ligule is attached immediately distal to the sporangia and is often obvious in the apical border of the sporophylls (Figs.3a,c,d,h-j). Trabeculae similar to those of *Isoetes* have also been observed in the sporangia of several species. According to Snigirevskaya (1989), the sporophylls of *Pleuromeia* are similar to *Isoetes* leaves having lost their distal sterile portion. Indeed, as suggested by this author, they had a tip which was longer than usually depicted but which dried and decayed in maturing cones. However, examination by one of us (L.G.-S.) of sporophylls of *P. sternbergii* and *P. rossica* in side view shows that they have a tip extending from the lamina without direction change and that this tip is a little longer than the border of the sporophylls in face view (Grauvogel-Stamm, 1999), but never as long as suggested by Snigirevskaja (1989).

Lycopsids with the same growth habit as *Pleuromeia* already existed in the Paleozoic: C*levelandodendron ohioensis* Chitaley and Pigg (1996) from the Late Devonian of Ohio, USA and *Chaloneria cormosa* Pigg and Rothwell (1983) from the Pennsylvanian of North America. *Clevelandodendron ohioensis* consists of 1.25 m tall unbranched plants covered with helically arranged leaf scars, and possessing a well defined bisporangiate cone containing trilete microspores and megaspores (Fig. 2a). Its partially preserved rhizomorph is said to bear "thick appendages". *Chaloneria cormosa* is also unbranched, and is 2 m tall with a bilaterally symmetrical cormose rhizomorph (Fig. 2b). Its reproductive organs consist of a terminal fertile zone up to 21 cm long, with alternating megasporangial and microsporangial regions resembling the sterile ones.

Meyen (1987) has suggested that *Pleuromeia* evolved from *Chaloneria*, and Stewart and Rothwell (1993) state: "*Chaloneria* seems to bridge the gap between the Upper Devonian treelike lycopsids with their cormose rhizomorphs and the cormose Triassic *Pleuromeia*". The discovery of *Clevelandodendron ohioensis* may indicate that a lineage comprising *Pleuromeia*-like plants already existed in the Late Devonian and was contemporaneous with the beginning of the radiation of arborescent lepidodendrids. This suggests that the di-

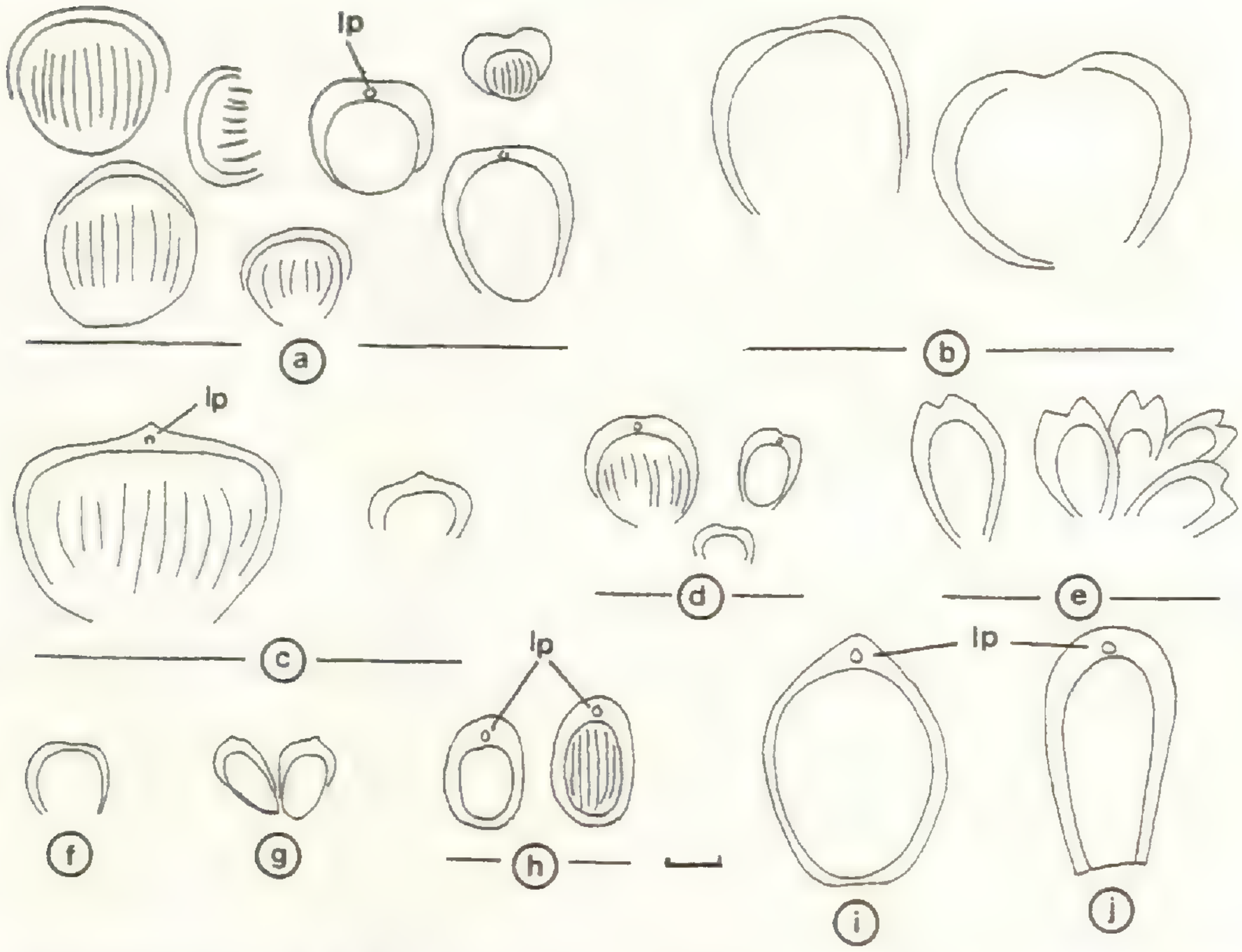

FIG. 3. Comparative shape of the sporophylls in *Pleuromeia*. Note the globose sporangium occupying most of the upper surface; note also the ligular pit (lp) distal to the sporangium, often visible in the narrow apical border of the sporophyll. (scale-bar, between h and i = 1cm). a—*P. sternbergii;* Lower Triassic of Germany (redrawn from Solms-Laubach, 1899; Potonié, 1904). b—*P.altinis;* Lower Triassic of North China (redrawn from Wang and Wang, 1989). c—*P. sternbergii;* Lower Triassic of North China (redrawn from Wang and Wang, 1989). d—*P. rossica;* Lower Triassic of Russia (redrawn from Neuburg, 1960; Dobruskina, 1982). e—*P. epicharis*; Lower Triassic of North China (redrawn from Wang and Wang, 1989, 1990). f—*P. patriformis;* Lower Triassic of North China (redrawn from Wang and Wang, 1989). g—*P. jiaochengensis;* Lower Triassic of North China (redrawn from Wang and Wang, 1982). h—*P. sanxiaensis;* early Middle Triassic of South China (redrawn from Meng, 1995, Fig. 3b,c). i—*P. marginulata;* early Middle Triassic of South China (redrawn from Meng, 1995, Fig. 3a). j—*P. hunanensis;* early Middle Triassic of South China (redrawn from Meng, 1995, Fig. 3d).

versification within the Isoetales was well established prior to the Carboniferous (Chitaley and Pigg, 1996). It may also question the statement of DiMichele and Bateman (1996) which suggests that the isoetalean line evolved from the lepidodendrids.

THE SPORES OF *PLEUROMEIA*.—Like the structure of the rhizomorphs, the ultrastructure of the spores of *Pleuromeia* appears to be phylogenetically informative, suggesting a close affinity with *Isoetes*.

The microspores of both genera, especially, show many ultrastructural similarities (Lugardon et al., 1997, 1999). This is all the more noteworthy since *Isoetes* microspores have a rather large number of distinctive characteristics which clearly differentiate them from all the other extant spore types, as stressed below. In order to underscore the similarities in ultrastructure and

correspondences of the homologous walls of *Pleuromeia* and *Isoetes* microspores, a description of both is given hereafter (Fig. 4).

Isoetes microspores are monolete spores easily recognizable, through light microscope, by an apparently bipartite sporoderm consisting of an ovoid, unadorned inner part wrapped in a wide, thicker, usually slightly ornamented outer part (Fig. 4a). The inner part is generally regarded as the exospore in morphological studies, while the outer part is variously interpreted and termed (perine, sexine. . .). TEM studies (Lugardon, 1973, 1980) have shown that these microspores possess three distinct aceto-resistant walls (Fig. 4b). The innermost one, which really represents the exospore, is a thin, almost plain-surfaced, bilayered wall that is characterized mainly by its aperture and by special areas of the wall called "laminated zones" (= "zones pluristrates" in the initial description of Lugardon, 1973). The aperture is distinguished by a simple reduction of the exospore thickness, without any other special modification. The laminated zones are small, appreciably thickened areas of the exospore arranged on both sides of the aperture, in which the exospore inner layer is tangentially cleft into several irregularly segmented laminae. The middle wall, that is called "para-exospore", consists of interconnected elements composing a spongy layer which is detached from the exospore all around the spores except in some points of the lateral regions of the proximal face; moreover this wall forms a high projection, usually divided apically into two lips, above the aperture. The outermost wall, which is termed perispore, is a rather thick, complex wall closely applied to the para-exospore surface. Ontogenetical fine studies (Lugardon, 1990; Tryon and Lugardon, 1991) showed that the para-exospore elements develop at the same time as the exospore outer layer, through simultaneous accumulation of the same sporopollenin on the exospore inner layer and on the tenuous framework of the para-exospore that is formed very early in the course of sporogenesis. Moreover, these studies showed that the perispore develops after the para-exospore and exospore completion, with materials of different chemical nature, like the typical perispore of homoporous fern spores (it is generally admitted that a genuine perispore is formed only in pteridophytes having a plasmodial tapetum; nevertheless, TEM studies proved that, besides the microspores of recent Isoetaceae, those of a number of Selaginellaceae, as well as the spores of rather numerous Lycopodiaceae, have a perispore comparable to that of the fern spores, although the lycopsids possess a secretory tapetum; Lugardon, 1990). Thus the wide sporoderm outer part of *Isoetes* microspores comprises two quite distinct components, i.e an uncommon structure, the para-exospore, that is ontogenetically related to the exospore, and a veritable perispore.

The microspores of *Pleuromeia* are trilete and rounded or rounded-triangular in equatorial outline (Fig. 4c). The morphological studies achieved with light and scanning electron microscopes (Neuburg, 1960; Yaroshenko, 1975) showed that these microspores have an outer envelope largely separated from a much thinner inner part often called inner body. The outer envelope appears spongy and faintly ornamented. The inner body is roughly smooth-surfaced and reveals under the light microscope, especially in specimens having lost

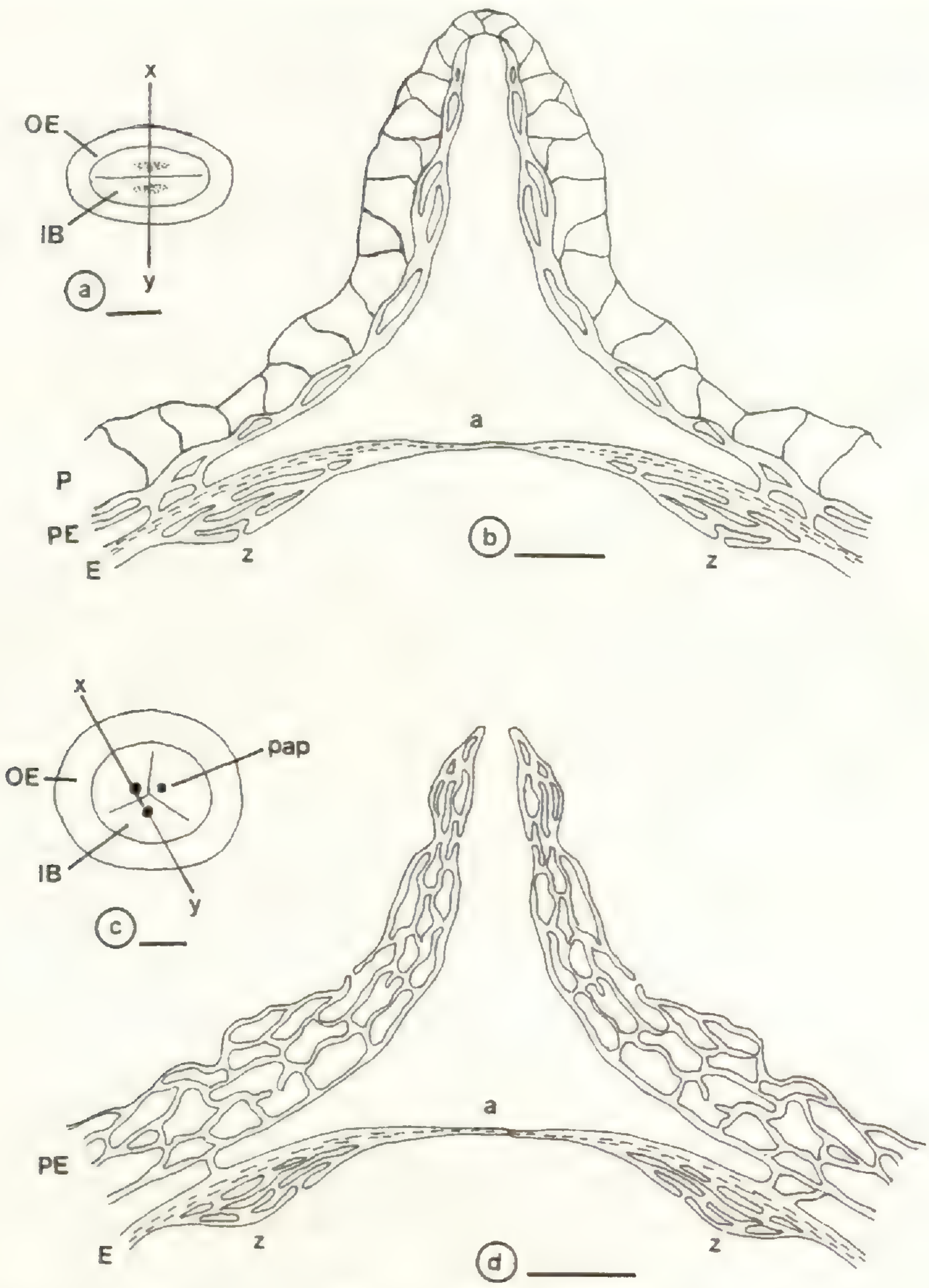

FIG. 4. Comparative ultrastructure of the apertural area in the microspores of *Isoetes* and *Pleuromeia* (scale-bar = 10μm in a, c and 2 μm in b, d). a—*Isoetes*—Diagrammatic representation of the microspore proximal face showing the outer envelope (OE) and the inner body (IB). The dotted spots on both sides of the monolete aperture mark the location of the laminated zones observed with TEM, but indiscernible in intact spores observed with light microscope. b—*Isoetes*. Ultrastructural features of the apertural area of a microspore sectionned along x-y, as shown in Fig. 4a. Note that there are three distinct resistant walls: the exospore (E) with the aperture (a) consisting of a thinner, non-folded wall portion, bordered with laminated zones (z); the para-exospore (PE) forming protruding lips above the aperture; the perispore (P) almost regularly thickened and unbroken above the apical para-exospore lips. c—*Pleuromeia*—Diagrammatic representation of the microspore proximal face showing the outer envelope (OE) and the inner body (IB) with three interradial papillae (pap) arranged between the arms of the trilete aperture and visible with light microscope only in particular conditions of preservation. d—*Pleuromeia*—Ultrastructural features of the microspore apertural area sectionned along x-y, as shown in Fig. 4c. Two distinct walls are preserved: the exospore (E) with the aperture (a) and laminated zones (z) similar to those in *Isoetes*; the para-exospore (PE) which is thicker but structurally analogous with that of *Isoetes* microspores.

the outer envelope, three rounded papillae arranged between the rays of the trilete mark near the proximal pole (Fig. 4c). Ultrastructural investigations in *Pleuromeia rossica* (Lugardon et al., 1997, 1999) and *P. sternbergii* (Grauvogel-Stamm and Lugardon, in prep.) proved that the inner body is a two-layered exospore, uniformly solid and thick except in the proximal region where it shows two notable characteristics (Fig. 4d). In the aperture area indeed, the exospore simply appears thinner without other noticeable differentiation, as in *Isoetes*. Secondly, in each of the three areas lying between the aperture rays, the exospore shows a well-defined laminated zone which is quite similar to those of *Isoetes* microspores, and which obviously corresponds to one of the three interradial papillae observed with light microscope. The outer envelope wholly consists of interconnected elements which show the same electron permeability and reactions to chemical reagents as the outer layer of the exospore, and are surely made up of the same sporopollenin. It is attached to the exospore in the peripheral regions of the proximal face. In the other spore regions, this outer envelope is usually separate from the exospore, and forms a well-marked protrusion divided apically into two lips above each of the three aperture rays.

Thus, the ultrastructural features of the exospore are very similar in *Pleuromeia* and *Isoetes* microspores, in spite of the fact that those of *Pleuromeia* are trilete whereas those of *Isoetes* are monolete. Likewise, the main features of the outer envelope of *Pleuromeia* microspores remarkably resemble those of the para-exospore in *Isoetes*, so that both walls obviously appear homologous, and can be equally called para-exospore. Besides the trilete or monolete condition, the only noteworthy differences lie in the lesser development of the para-exospore and the presence of an extra wall, the perispore, in *Isoetes*.

The combination of the exospore and para-exospore features which is quite identical in *Isoetes* and *Pleuromeia* microspores is also that of the dispersed spore genera *Endosporites* and *Aratrisporites* which are respectively produced by *Chaloneria/Polysporia* and *Annalepis* (Lugardon et al. 2000b, Grauvogel-Stamm and Lugardon, in prep.). The three interradial papillae have been clearly shown in *Endosporites* by Brack and Taylor (1972). This combination of features differentiates these microspores from all the other spore types of extant, and probably fossil, pteridophytes. The aperture consisting of a simply thinner, unfolded exospore area appears unique, and contrasts with the well-marked prominent fold showing various outlines and more or less intricate structures that characterizes the aperture of most pteridophyte spores, including the microspores of Selaginellaceae (Lugardon, 1972, 1980, 1986; Tryon and Lugardon, 1991). Moreover, although the laminated zones are present in the microspores of many Selaginellaceae, they are totally missing in a rather important number of species of this large family (Tryon and Lugardon, 1991: Figs. 231.21, 25, 26,28). Likewise, the para-exospore appears to exist only in very few species of Selaginellaceae in most of which the exospore is devoid of laminated zones, and the para-exospore is almost solid and does not form a sharp protrusion above the aperture (Tryon and Lugardon, 1991: Figs. 231. 26, 28). Among the recent and fossil lycopsids in which the spore ultrastructure

is known to date, the microspores which most resemble those of *Pleuromeia* and *Isoetes* are those of the present *Selaginella selaginoides* (Lugardon, 1972) and those from the Pennsylvanian lycophyte cone *Selaginellites crassicinctus* (Taylor and Taylor, 1990). In both, indeed, the microspores have an exospore with laminated zones and a fairly spongy para-exospore forming prominent lips above the aperture rays. However, these spores are unambiguously distinguishable by the specially narrow, strongly marked apertural fold of the exospore (known only in Selaginellaceae microspores among recent pteridophyte spores, as it can be seen in Tryon and Lugardon, 1991). Thus, not only the microspores of *Isoetes* and *Pleuromeia,* as well as those of *Annalepis* (*Aratrisporites*) and *Chaloneria/Polysporia* (*Endosporites*), show very close ultrastructural similarities, but also they differ markedly from the spores of all the other pteridophyte groups, including the especially variously structured microspores of Selaginellaceae.

It is regrettable that there are no adequate data on the fine features of the microspores of other fossil rhizomorphic lycopsids, particularly the lepidodendrids, which could be compared to those of *Pleuromeia*. The different TEM studies on the lepidodendrid microspores *Lycospora,* achieved by Thomas (1988), Taylor (1990), Hemsley and Scott (1991), Scott and Hemsley (1993), suggest that these microspores also have a thin exospore partly separated from a thicker outer envelope presumably equivalent to a para-exospore. However the *Lycospora* studied by these authors, like those presently investigated (unpublished observations, B.L.), are rather poorly preserved and have not clearly shown the ultrastructural characteristics of their apertural area which, very likely, would provide significant information on the relationships of the lepidodendrids with the other lycopsid lineages.

The megaspores of *Pleuromeia* are also trilete with a slight ornamentation and a spongy aspect rather comparable to that of the microspores (Zhelezkova, 1985; Marcinkiewicz and Zhelezkova, 1992). Ultrastructurally (Lugardon et al., 2000a; Grauvogel-Stamm and Lugardon, in prep.), their exospore consits of a very thin, solid inner layer and a thick spongy outer layer which constitutes most of the wall and is usually divided by a tangential gap in its innermost area, except near the aperture. These features, as well as those of the aperture, are comparable to those of the *Isoetes* megaspores (Lugardon, 1986; Taylor, 1993 ; Tryon and Lugardon, 1991). However they are also analogous to those of all the other, recent or fossil, lycopsid megaspores, so that they do not provide any special phylogenetic information. Nevertheless, the ultrastructural study of especially well-preserved megaspores of *P. rossica* (Lugardon et al., 2000a) demonstrates that the spongy outer layer consists of elements which usually show a rod-like shape, are mostly arranged parallel to the wall surface and are rather widely spaced in moderately crushed spores. These features of the exospore outer layer, which are quite obvious and unvarying throughout the wall in those *P. rossica* megaspores, precisely represent the few characteristics regarded as distinctive of the isoetalean megaspores (Kovach, 1994). Thus the megaspores indicate also, although in a less demonstrative way than

the microspores, the relation joining the Triassic *Pleuromeia* to the living *Isoetes*.

Furthermore, the exospore inner layer of *Pleuromeia* megaspores includes, along both sides of the three apertural arms, a number of laminated zones which are structurally quite similar to those of the microspores. Such laminated zones, that have not been observed in *Isoetes* megaspores, undoubtedly correspond to the "papillae, pustules, cushions, nipple-like projections. . ." described in several morphological studies of fossil lycopsid megaspores. In particular, rather abundant papillae, i.e. 15–20 on either side of each apertural ray, can be observed with LM in the megaspores of *P. sternbergii* (Grauvogel-Stamm and Lugardon, in prep.). However, for the moment these structures do not provide any reliable information on the affinities of *Pleuromeia* since their distribution among the lycopsids is not clearly characterized.

The Triassic lycopsid *Annalepis* and related taxa: comparative study.

Besides *Pleuromeia*, there is another noteworthy Triassic lycopsid, *Annalepis* Fliche, which has long been misunderstood. The type-species, *A. zeilleri* Fliche from the Middle and Upper Triassic of eastern France, is represented by isolated "scales" described by Fliche (1910) who noticed their resemblance to seed scales of the conifer *Araucaria* but finally placed them as an incertae sedis. Grauvogel-Stamm and Duringer (1983) showed that the scales of *A. zeilleri* are sporophylls belonging to the lycopsids and containing microspores assignable to *Aratrisporites*. Such sporophylls are often termed "scales" in descriptions, likely on the basis of their shape and maybe on the analogy of the wording used in the conifer seed scales. Undescribed new material from the Ladinian (late Middle Triassic) of Germany now allows to give some further details, particularly about the rest of the plant (Grauvogel-Stamm and Lugardon, in preparation).

Growth habit and reproductive organs of *Annalepis zeilleri* Fliche.—The sporophylls of *A. zeilleri* are preserved as compressions having 2.5–5 cm long and being slightly trapezoidal in shape (Fig. 5a, 6a). They consist of a long and wide proximal portion flaring abruptly at the base which is somewhat thickened, widening distally and extending by a thick and short, roughly triangular distal portion ending with a small pointed tip. The presence of a transverse wide groove followed by a bulge, distal to the sporangium, between the proximal and distal portions in the sporophylls in compression and adaxial view, indicates that their junction was more or less at right-angle. Indeed, the groove corresponds to the zone of juncture of the two portions and represents exactly the place where the distal portion was straightened up (Fig. 5d-e). This right-angled juncture remained partly preserved and appears as a groove because of the thickness of this zone while the thinner distal portion has been folded back by the sediment pressure and appears in the same plane as the proximal portion in the fossilized sporophylls. Sometimes the groove+bulge appears as a transversal fold (Fig. 5f). Thus, when the sporophylls were packed

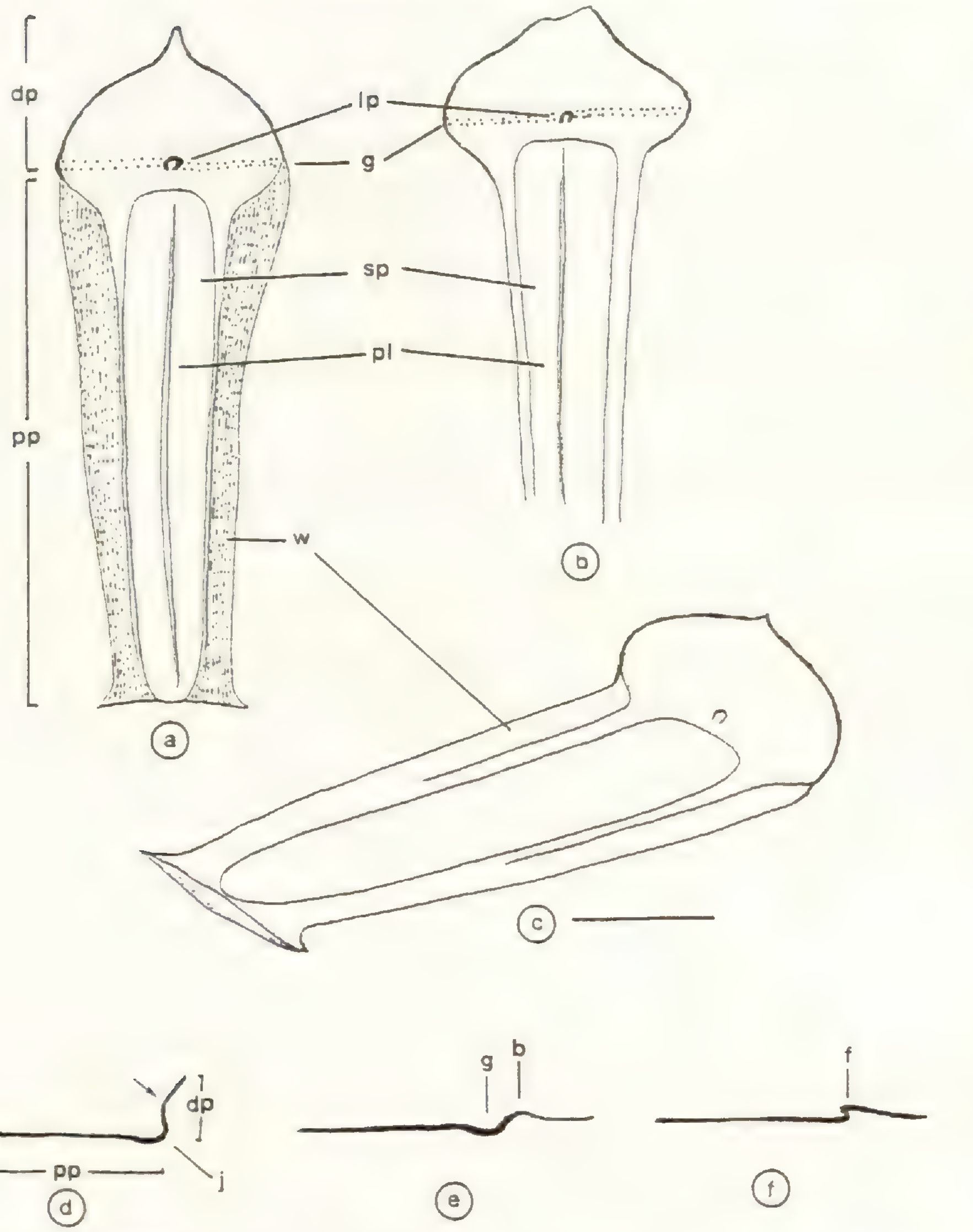

FIG. 5. The sporophyll of *Annalepis zeilleri* Fliche in adaxial view (a-c): (a) in compression, entire; (b) in compression but lacking the lateral wings; (c) in life condition (modified from Grauvogel-Stamm and Duringer, 1983) (scale-bar = 1 cm). Additional diagrammatic representations of sporophylls in side view (d-f), illustrating how the tranverse groove and either bulge or fold were formed in the junction zone between the proximal and distal portions of the sporophyll during the course of fossilization: (d) sporophyll in life condition, with both portions roughly perpendicular to each other (the arrow indicates the direction of flattening during fossilization); (e) flattened sporophyll with a bulge distal to the transverse groove; (f) flattened sporophyll showing a small fold above the groove. (pp = proximal portion; dp = distal portion, upturned in life position as in c and d; g = transverse groove; b = transverse bulge; f = transverse fold; j = thickened junction zone; sp = sporangium; w = wing; lp = ligular pit; pl = rod-like placenta, located on the abaxial side but often visible on the adaxial side of the sporophylls in compression, especially when the sporangium is empty.

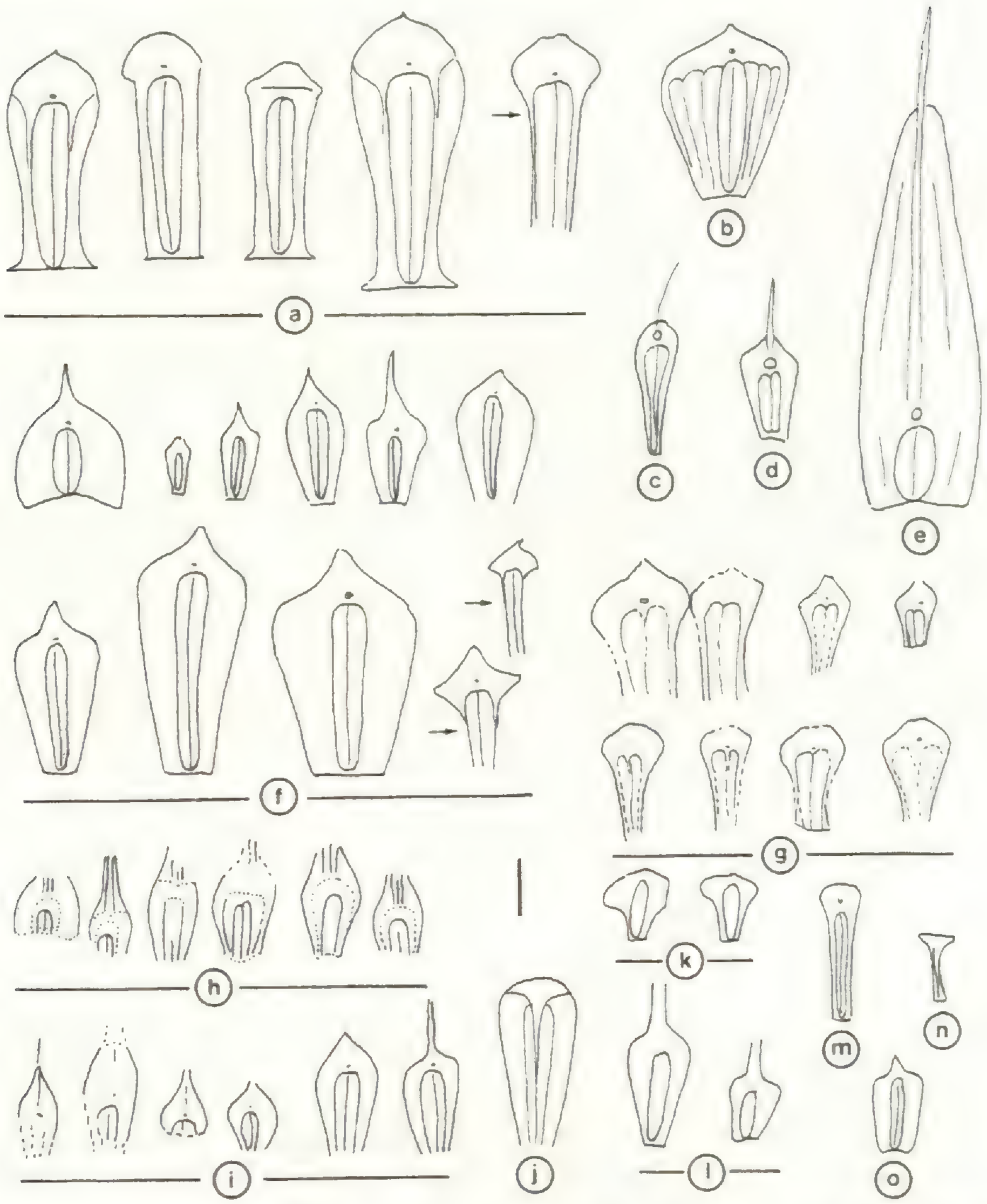

FIG. 6. Comparative morphology of the sporophylls of the *Annalepis*-type and related genera. (Scale-bar = 1 cm). a—*Annalepis zeilleri* Fliche (from Grauvogel-Stamm and Duringer, 1983). The arrow shows a scale having lost its lateral wings. b—*A. latiloba* Meng (redrawn from Meng, 1998). c—*A. angusta* Meng (redrawn from Meng, 1995). d—*A. sangzhiensis* Meng (redrawn from Meng, 1995). e—*A. brevicystis* Meng (redrawn from Meng, 1998). f—*Annalepis* sp. (unpublished). Note the range of variation in shape, size and apex length. The arrows indicate two scales having lost their lateral wings. g—*Isoetes ermayinensis* Wang (redrawn from Wang, 1991). h—*Tomiostrobus radiatus* Neuburg (redrawn from Dobruskina, 1985). i—*Tomiostrobus belozerovii*, *T. fusiformis*, *T. bulbosus*, *T. convexus*, *T. gorskyi* and *T. migayi*, from left to right (redrawn from Sadovnikov, 1982). j—*Lepacyclotes ellipticus* Emmons (redrawn from Fontaine, 1900). k—*Cylostrobus sydneyensis* Helby and Martin (redrawn from Helby and Martin, 1965). l—*Skilliostrobus australis* Ash (redrawn from Ash 1979). m—*Isoetites daharensis* Barale (redrawn from Barale, 1999). n—*Isoetites horridus* Brown from the Tertiary of North America (redrawn from Hickey, 1977). o—*Isoetites choffatii* (Saporta) (redrawn from Teixeira, 1948) .

together in the cones, their proximal portion was perpendicular to the cone axis whereas their distal part was upturned and more or less parallel to this axis (Fig. 5c). The adaxial surface of the proximal portion was occupied, in the middle and on the whole length, by a long, oval and well-delimited sporangium. The borders of the proximal portion which project on both sides of the sporangium appear to be membranous, as also noticed by Fliche (1910). The boundary between the median part bearing the sporangium and its membranous borders is not always very clear but it is particularly obvious when the imprints are covered with coaly organic matter which is thinner on the borders and therefore less dark than on the other parts of the scales. These membranous borders, called wings or alae according to the authors, likely tended to fall or to be damaged. In some specimens they are partly detached and in others they are completely missing (Fig. 5b), so that it appears clearly that the proximal part is widened distally and that the wings are restricted to the area below this distal widening (see Grauvogel-Stamm and Duringer, 1983; compare figs. 2 and 3 of Pl. 4). The long oval sporangium seems divided by a longitudinal midline which likely corresponds, as in *Isoetes* (Hall, 1971), to the trace of a rod-like placenta which was located on the abaxial side of the proximal portion of the sporophyll and to which the sporangium wall was attached. The presence of a ligule, which was not recognized when Grauvogel-Stamm and Duringer (1983) described these scales, is clear but this ligule is usually decayed and represented only by its attachment point, which is located immediately distal to the sporangium.

Undescribed new material from the Ladinian (late Middle Triassic) of Germany shows that the distal portion of these sporophylls was fleshy. The coalified organic matter covering their imprint is thick and the cell imprints are usually quite visible on its brilliant surface. These sporophylls were arranged in cones reaching at least 8–10 cm in diameter. The occurrence, together with these scales and cones, of several rounded furrowed rhizomorphs, probably 4-lobed, each still attached to a stem (10 cm long preserved) which is covered with oval leaf-scars (the leaves are unknown) is informative since it shows that this plant had the same growth habit as *Pleuromeia* and consisted of an unbranched monopodial axis with a bilaterally symmetrical rooting base and a large, terminal and compact cone (Fig. 7a). The impressive and numerous roots, 3–7 mm wide and more than 30 cm long, which are still attached to the rhizomorphs, may indicate that this plant was robust and strongly anchored. As shown by Grauvogel-Stamm and Duringer (1983), the sporophylls of the cones were monosporangiate, containing either monolete microspores (*Aratrisporites* Leschik) or trilete megaspores (*Tenellisporites marcinkiewiczae* Reinhardt and Fricke). No specimens offer evidence as to whether the cones were bisporangiate or monosporangiate. The ultrastructural features of the monolete microspores are comparable to those of *Pleuromeia* and *Isoetes* (Grauvogel-Stamm and Lugardon, in preparation).

THE OTHER *ANNALEPIS* SPECIES.—Five further species of *Annalepis* have been described from the Anisian (early Middle Triassic) of South China: *A. furo-*

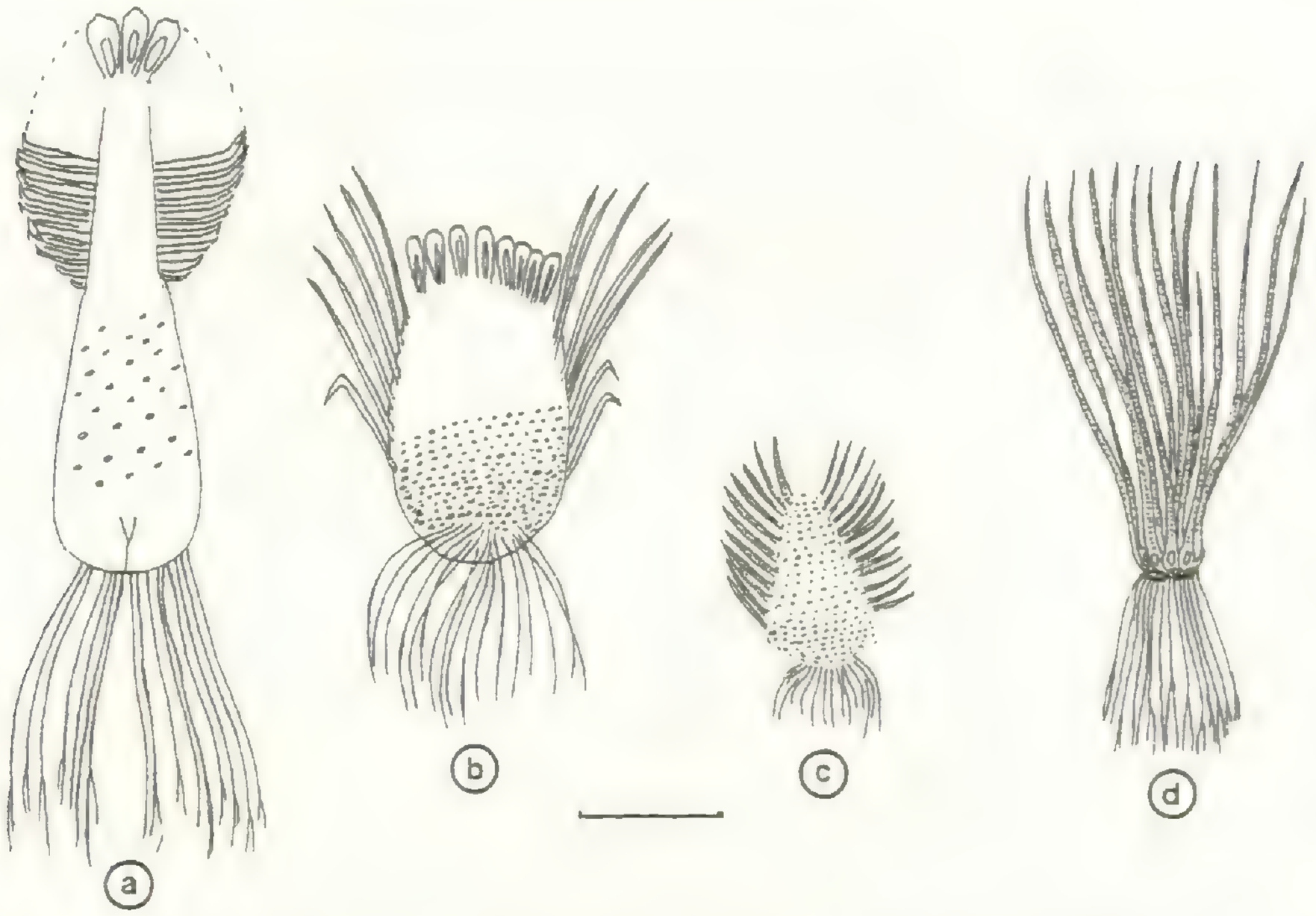

FIG. 7. Comparative growth habit of *Annalepis, Isoetites* and *Isoetes*. (Scale-bar = 5 cm). a—*Annalepis zeilleri* Fliche. Note the apical massive cone and the basal rounded rhizomorph bearing numerous long roots, more than 30 cm long, arranged in two sets on both sides of the furrow. The leaves are unknown but their scars cover helically the stem. Ladinian of Germany (unpublished). b—*Isoetites daharensis* Barale. Note the basal rounded rhizomorph and the stocky stem covered with helically arranged elongated sterile leaves and distal *Annalepis*-type sporophylls which likely were packed in a cone as suggested by the specimens figured by Barale, which are at the origin of this modified reconstruction. Lower Cretaceous of Tunisia. (modified from Barale, 1999). c—*Isoetites phyllophila* Skog et al., showing a conical, elongate corm covered with numerous helically arranged lacunate sterile leaves. Mid-Cretaceous from North America. (redrawn from Skog et al., 1992). d—*Isoetes boryana*, having 20–30 lacunate leaves 10–20 cm long (redrawn from Motelay and Vendryès, 1882)

ngqiaoensis Meng, *A. latiloba* Meng (Fig. 6b), *A. angusta* Meng (Fig. 6c), *A. sangzhiensis* Meng (Fig. 6d) and *A. brevicystis* Meng (Fig. 6e). The last three species are shown to have a long, narrow leaf tip emerging abruply from a rounded apex (Meng, 1995, 1996, 1998, 2000). The sporophylls representing *A. brevicystis* are by far bigger than the others and greatly differ in shape. Meng (1998) moreover described several further sporophylls with a long leaf tip containing transverse partitions which he assigned to *A. zeilleri* but which probably correspond to another species. Indeed none of the specimens corresponding to the original material of *A. zeilleri* described from the Ladinian (late Middle Triassic) of France show such features (Grauvogel-Stamm and Duringer, 1983). In all the sporophylls described by Meng, the sporangium is tongue-shaped and is longitudinally divided by a midline. Unfortunately none of the photographic illustrations of the Chinese specimens clearly shows the structure of these sporophylls, such as it is shown in the rough reconstructions figured by Meng (1995, 1998, 2000).

Furthermore, an assemblage of numerous undescribed sporophylls from the Ladinian (late Middle Triassic) of Germany which is here provisionally as-

signed to *Annalepis* sp. (Fig. 6f), represents a further new species which is still under study (Grauvogel-Stamm and Kelber, in preparation). Indeed, these sporophylls which originally have been attributed to *A. zeilleri* by Kelber and Hansch (1995) contain features which distinguish them from this species. They show a great variability in shape, size and apex length, which probably depends on their place within the cone. Transverse partitions have never been observed in any of the long apex of these sporophylls. However, they seem to have lateral membranous borders as in *A. zeilleri* (Fig. 8a,b) and the tongue-shaped sporangium shows a longitudinal midline as in the other *Annalepis*-like sporophylls.

In spite of a roughly similar organization, the sporophylls of *Pleuromeia* and *Annalepis* differ in several respects. Their general outline and the shape of their sporangium are quite different. The wings bordering the sporophyll proximal part and the longitudinal midline running along the sporangium in *Annalepis* are missing in *Pleuromeia*. In contrast to the sporophylls of *Annalepis* that are clearly differentiated into a proximal and a distal portions which were very likely more or less perpendicular to each other in lifetime, those of *Pleuromeia* do not show such a morphological partition and are approximately on the same plane from the base to the apex. Moreover, in *Pleuromeia* the microspores and megaspores are trilete whereas in *Annalepis* only the megaspores are trilete while the microspores are monolete (Grauvogel-Stamm and Duringer, 1983), as those of Isoetes.

THE ALLIED GENERA: FROM *ANNALEPIS* TO *ISOETITES*.—While it is easy to recognize a sporophyll as belonging to the genus *Pleuromeia,* there are difficulties in identifying those of the *Annalepis*-type. Most of the latter have been long misunderstood, being first regarded as araucarian seed scales and therefore often assigned to the genera *Araucarites* Presl or *Pseudoaraucarites* Vladimirovitch (Dobruskina, 1985). Now, eventhough they are regarded as lycopsids, these sporophylls are assigned to different genera as *Tomiostrobus* Neuburg, *Skilliostrobus* Ash, *Cylostrobus* Helby and Martin and *Lepacyclotes* Emmons, and classified, together with *Annalepis,* as satellite-taxa in the Pleuromeiaceae (Thomas and Brack-Hanes, 1984). However their taxonomic position is greatly in flux. Dobruskina (1985) stressed the similarity between *Annalepis* and *Tomiostrobus* whereas Retallack (1997) synonymized *Annalepis* with *Lepacyclotes*. Moreover Sadovnikov (1982) and Retallack (1997) synonymized *Skilliostrobus* with *Tomiostrobus*. Nevertheless, since the genus *Annalepis,* particularly the type-species *A. zeilleri,* is the best known, it is here regarded as the type of this kind of sporophyll. *Lepacyclotes* was described earlier by Emmons (1856) but the precise structure and the in situ spores have never been studied. Other taxa for which the structure and the shape of the sporophylls are unknown include *Lycostrobus scotti* Nathorst and *Austrostrobus ornatum* Morbelli and Petriella. As for *Lycostrobus chinleana* Daugherty from the Triassic of southwestern USA, it has proven to be an equisetalean reproductive organ (Grauvogel-Stamm and Ash, 1999) and not a lycopsid, as previously believed, or an arborescent quillwort, as recently asserted by Retallack (1997).

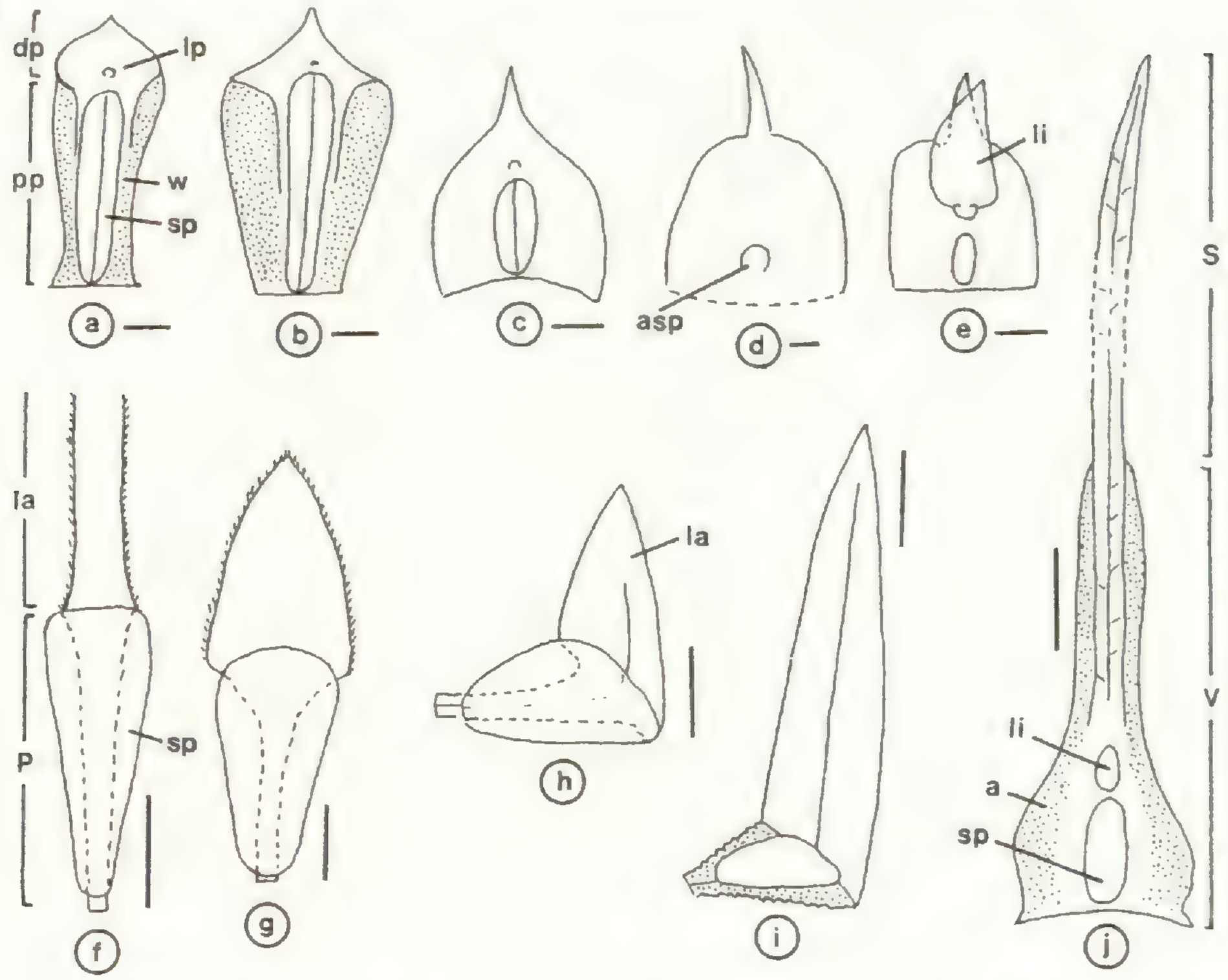

FIG. 8. Comparative morphology and terminology of the sporophylls of *Annalepis*, Lepidodendrids and *Isoetes* in adaxial views. (scale-bar = 5mm, except in d,e = 1mm). a—*Annalepis zeilleri* Fliche (pp, proximal portion; dp, distal portion; lp, ligular pit; sp, sporangium; w, wing). Note the clearly delimited wings (stippled) of the proximal portion (modified from Grauvogel-Stamm and Duringer, 1983). b, c—*Annalepis* sp. (undescribed): two sporophylls within the range of variation. The wings (stippled) are clearly delimited in b, while they are not in c. d—*Isoetes coromandelina* Lin. Fils: a scale-leaf with an aborted sporangium (asp) (redrawn from Srivastava and Wagai, 1996, Pl.4 Fig. 1). e—*Isoetes* sp.: a leaf primordium with the developing ligule (li) and sporangium (redrawn from Kubitzki and Borchert, 1964). f—*Cyclostigma kiltorkense* Haughton (p, pedicel; la, lamina; sp, sporangium). Note the narrow pedicel slightly widened distally below the sporangium and the distal lamina that is linear lanceolate, up to 8 cm long (modified from Chaloner, 1968). g—*Lepidostrobus bohdarowiczii* Bochenski. The narrow pedicel is here markedly widened distally (modified from Bochenski, 1936). h—*Lepidostrobus jenneyi* (White) Abbott. Sporophyll showing a proximal pedicel below the sporangium and an apically directed lamina (la). Due to its strong distal widening, the pedicel has been described T-shaped by Abbott (modified from Abbott, 1963). i—*Lepidocarpopsis lanceolatus* (Lindley and Hutton) Abbott. The pedicel clearly bears well-developed wings (stippled) and therefore exceeds the width of the sporangium (modified from Abbott, 1963). j—*Isoetes triquetra* A. Braun. Sporophyll showing the vagina (v) with lateral alae (a, stippled), sporangium (sp), ligula (li), and the terminal subula (s) 3,4 cm long (modified from Kubitzki and Borchert, 1964; terminology after Hickey 1985).

Since the precise structure and shape of the sporophylls are essential for identifying and comparing these lycopsids, only the genera for which these features are known with considerable accuracy and which resemble the *Annalepis*-type sporophylls are listed in Table 1 and shown in Fig. 6, Some *Isoetites* are also listed but their inclusion raises some questions on the exact interpretation of this fossil genus, as commented below.

TABLE 1. The *Annalipis*-like sporophylls. Data taken from the following sources: [1]Fliche (1910); [2]Meng (1995); [3]Meng (1998); [4]Meng (2000); [5]Dobruskina (1985); [6]Sadovnikov (1982); [7]Wang (1991); [8]Emmons (1856); [9]Ash (1979); [10]Helby and Martin (1965); [11]Teixeira (1948); [12]Barale (1999); [13]Brown (1958), Hickey (1977).

Taxon	Age	Occurrence	Figure
Annalepis zeilleri[1] (type-species)	Middle Triassic	NE France; Germany	Fig. 6a
A. furongqiaoensis[4]	Middle Triassic	Southern China	
A. latiloba[3]	Middle Triassic	Southern China	Fig. 6b
A. angusta[2]	Middle Triassic	Southern China	Fig. 6c
A. sangzhiensis[2]	Middle Triassic	Southern China	Fig. 6d
A. brevicystis[2]	Middle Triassic	Southern China	Fig. 6e
Annalepis sp. (undescribed)	Middle Triassic	Germany	Fig. 6f
Isoetes ermayinensis[7]	Middle Triassic	North China	Fig. 6g
Tomiostrobus radiatus[5] (type-species)	Lower Triassic	South Siberia, Russia	Fig. 6h
T. belozerovii[6]	Lower Triassic	East Siberia, Russia	Fig. 6i
T. fusiformis[6]	Lower Triassic	East Siberia, Russia	Fig. 6i
T. bulbosus[6]	Lower Triassic	East Siberia, Russia	Fig. 6i
T. gorskyii[6]	Lower Triassic	East Siberia, Russia	Fig. 6i
T. migayi[6]	Lower Triassic	East Siberia, Russia	Fig. 6i
T. convexus[6]	Lower Triassic	East Siberia, Russia	Fig. 6i
Lepacyclotes circularis[8] (type-species), syn. *L. ellipticus*[8]	Upper Triassic	North Carolina, USA	Fig. 6j
Cylostrobus sydneyensis[10] (type-species)	Lower Triassic	Australia	Fig. 6k
Skilliostrobus australis[9] (type-species)	Lower Triassic	Australia	Fig. 6l
Isoetites daharensis[12]	Lower Cretaceous	Southern Tunisia	Fig. 6m
Isoetites horridus[13]	Tertiary	North America	Fig. 6n
Isoetites choffati[11]	Lower Cretaceous	Portugal	Fig. 6o

The sporophylls characterizing these taxa share a remarkable similarity with *Annalepis*. They have a comparable shape and structure (Fig. 6a-o). Most of them (*Annalepis, Tomiostrobus, Isoetes ermayinensis, Skilliostrobus, Cylostrobus*) contain microspores assignable to *Aratrisporites* (Balme, 1995). In all, the sporangium is elongate-oval and shows a longitudinal midline which has been variously interpreted. In *Annalepis zeilleri,* Grauvogel-Stamm and Duringer (1983) suggested that it might represent the trace of the vascular bundle (Fig. 6a). In *Isoetites daharensis* (Fig. 6m), this line, which is obvious too and quite resembles that of *Annalepis,* has been interpreted as being the internal boundary of the velum (Barale, 1999). In *Isoetes ermayinensis* (Fig. 6g) it has been regarded as a strong keel or a vascular bundle (Wang, 1991). In *Tomiostrobus* (Figs.6h,i), Sadovnikov (1982) interpreted it as a keel whereas Dobruskina (1985) regarded it as the vascular bundle. Fontaine (1900) also regarded it as a keel in *Lepacyclotes ellipticus* (Fig. 6j). The fact that this line appears invariably the same in the different scales indicates that it is a characteristic feature of this kind of sporophyll and that it has the same significance in all. A comparison with *Isoetes* shows that it does not represent the velum which is a thin membrane covering partially or entirely the sporangium and which never has a longitudinal slit-like free edge in the middle. In contrast, the longitudinal midline more likely indicates that, as in *Isoetes* (Hall, 1971), a rod-

like placenta including the vascular bundle existed on the abaxial side of these sporophylls, to which the sporangium wall was attached, and that it became apparent on the adaxial side of the compressions particularly when the sporangium was empty. According to J. Hickey (pers. com), in *Isoetes* this placenta is represented by a pad of vascularized tissue which often intrudes into the sporangium and runs lengthwise. Hall (1971) moreover noticed that in dried specimens of *I. tenuifolia* and *I. pitotii*, the rod-like shaped placenta shrinks and leaves an especially deep and profound groove.

In some specimens of *Tomiostrobus* (Figs. 6h,i) and *Skilliostrobus* (Fig. 6l) which one of us (L. G.-S.) observed, and according to the illustrations of *Isoetes ermayinensis* (Fig. 6g) and *Cylostrobus sydneyensis* (Fig. 6k), most of the scales seem to have a proximal portion with lateral wings, as in *A. zeilleri* (Fig. 6a). These lateral wings are clearly shown in the transverse section of the sporophyll of *Tomiostrobus* figured by Sadovnikov (1982, Fig. 2, at right), though not noticed by him. They are comparable to those shown in transverse sections of *Isoetes* sporophylls (Pitot, 1959; Hall, 1971). In some specimens the wings seem to be missing or partly destroyed, as in *Skilliostrobus* (see Ash, 1979, Figs. 7 A–D) or *I. ermayinensis* (see Wang, 1991, Pl. 1 figs. 9–11, 13, 14). However the question of the wings would require further investigations since their presence has not been noticed in most of the sporophylls listed in Table 1.

The long, oval and contiguous depressions, often visible on both sides of the sporangium in most of the scales, such as *Annalepis* sp., *A. zeilleri*, *A. latiloba* and *Lepacyclotes circularis*, correspond to the imprint of the sporangia of the underlying and overlying sporophylls when they were packed together in the cone.

The wide transverse groove visible at the junction of the proximal and distal, thick portions of the scales of *L. circularis*, as seen in adaxial view and as figured by Bock (1969, Figs. 93–94), is quite similar to that observed in *A. zeilleri* (Grauvogel-Stamm and Duringer, 1983, Pl. 4 figs. 2,3); this strongly suggests that the proximal and distal portions were originally more or less perpendicular to each other in *Lepacyclotes* too. The sporophylls of *Tomiostrobus* which one of us (L.G.-S.) observed, also show that the junction of the proximal and distal portions was more or less right-angled. Likewise, in *Annalepis* sp., the distal portion also seems to have been upturned. Also Meng (1998) noticed the presence of a transverse ridge in *A. latiloba* which suggests that the sporophyll distal portion was more or less perpendicular to the proximal one.

However various interpretations and/or misinterpretations have occurred because these sporophylls often have a more or less blunt, seemingly incomplete apex and/or because the scale tip has been considered as being the ligule, making the interpretation of this area confusing. Moreover, some of these sporophylls have been regarded as having lost their leafy distal portion. The sporophylls of *Isoetes ermayinensis* Wang (Fig. 6g) are so similar to those of *A. zeilleri* (Fig. 6a) that the inclusion of that taxon in *Isoetes* is surprising. In fact, Wang (1991) interpreted the numerous, associated elongate leaf imprints as their detached apical parts. These imprints contain transverse partitions that

he supposed to be collapsed air chambers, similar to those of *Isoetes*. However no specimens show them in organic connection. A similar leaf imprint found associated with sporophylls of *A. brevicystis* in the Anisian of South China has also been regarded as their tip (Meng, 1996, Pl. 2 Fig. 14). Sterile partitioned leaves have never been found associated with *A. zeilleri* from the Triassic of eastern France (Grauvogel-Stamm and Duringer, 1983). Retallack (1997) noticed that Wang's sporophylls of *I. ermayinensis* are "unlikely to have borne the wider undulose leaf fragments on their tips as in *Isoetes*" and reassigned *I. ermayinensis* to the genus *Lepacyclotes*. Likewise, the reassignment of *Lepacyclotes circularis* and *L. ellipticus* to the genus *Isoetites,* as proposed by Brown (1958), does not seem to be justified and has been questioned by Chaloner (1967) and Bock (1969) since these scales have a short and well delimited triangular apex and are devoid of any traces of a long sterile leafy portion comparable to that of *Isoetes*. Skog and Hill (1992) also questioned this reassignment and noticed the resemblance between *Lepacyclotes circularis* and *Annalepis*. Among the fossils attributed to the genus *Isoetites,* there are also isolated scales with a small pointed apical tip resembling the scales of *Annalepis,* which have been supposed to be the fertile base of *Isoetes* leaves. It is the case, especially, of *Isoetites horridus* (Fig. 6n) from the Tertiary of North America (Brown 1958, Figs. 1,3; 1962, Pl. 9 Fig. 1) and *I. choffati* Saporta (Fig. 6o) from the Cretaceous of Portugal (Teixeira, 1948, Pl. XXVI figs. 7–8). However the small apical tip is not preserved in some specimens, as those of *I. horridus* from the early Tertiary of Dakota figured by Hickey (1977, Pl. 1 figs. 6,7) and described as being dumb-bell shaped by Collinson (1991). Moreover, according to the illustration of Collinson (1991, Fig. 7.3h), which shows a circlet of such supposed fertile leaf bases, one can see that Hickey (1977) represented these sporophylls upside down and that the narrower extremity corresponds to the base while the broader one corresponds to the distal extremity to which the long leafy part was supposed to be attached. It is noteworthy that this circlet closely resembles those consisting of *Annalepis* sporophylls. Since the leaf tip is often missing in the *Isoetites* remains, Collinson (1991) suggested that this tip was deciduous in the fossil genus. In *Isoetes,* the long leafy part is usually not deciduous, except in some terrestrial species where the distal portion of the leaves erodes away leaving only a leaf base, typically with a sporangium (J. Hickey, pers. com.).

However, the fossils attributed to *Isoetites* call for several remarks. An overview of these fossils shows that the precise shape and structure of the fertile parts are usually poorly described and illustrated and that their relationship with the sterile leaves is not clear. For most taxa, the fertile and sterile parts are not shown in organic connection and several rhizomorphs and stems seem to contain only sterile leaves, e.g., *Isoetites phyllophila* (Skog et al., 1992), *I. horridus* (Brown, 1962; Hickey, 1977), *I. indicus* (Bose and Banerji, 1984), *I. serratus* (Brown, 1939), *I. rolandii* (Ash and Pigg, 1991) and *Cylomeia* (White, 1981) which is now reassigned to *Isoetes beestonii* (Retallack, 1997). It is surprising that none of them shows any trace of a sporangium at the base of the leaves. Likewise, the late Cretaceous and Tertiary specimens assigned to *Is-*

oetites by Pigg (2001) do not show clearly if there were sporangia at the base of the leaves. Long sterile leaves, without partitions, still attached to an axis have also been found associated with the sporophylls of *Annalepis* sp. in the Triassic of Germany and have been assigned to *Isoetites* sp. (Kelber and Hansch, 1995). In *Isoetes*, a great number of leaves are usually fertile (Jermy, 1990) and when once the plant becomes fertile, all subsequently produced leaves will either be fertile or at least initiate a sporangium (J. Hickey, pers. com.). During the ontogeny of the *Isoetes* leaves, the sporangium develops very early (Fig. 8e), is already discernible in the leaf primordia (Bhambie, 1963; Kubitzki and Borchert, 1964), and even the vegetative leaves have an aborted sporangium at the base (Pitot, 1959; Rauh and Falk, 1959; Kubitzki and Borchert, 1964). Thus the real nature of many *Isoetites* leaves remains quite ambiguous. Moreover, some of the described *Isoetites* species do not represent lycopsids. According to Skog and Hill (1992), *Isoetites gramineus* (Ward) Bock more likely is an osmundaceous fern and *I. bulbosus* Drinnan and Chambers more probably corresponds to "pentoxylalean fertile short shoots".

Likewise, the interpretations about the scales of *Isoetites* having a small tip and resembling the *Annalepis* sporophylls are still questionable. Do they represent fertile leaf bases which have lost their leafy extremity, and is the small tip visible in some of them actually a ligule? In *Isoetes,* the ligule is a delicate ephemeral parenchymatous flap which is mostly deciduous and tends to disappear at maturity (Pitot, 1959; Bhambie, 1963; Goswami, 1976; Kott and Britton, 1985). Therefore, it seems unlikely that the ligule would persist if the long leafy extremity of the sporophyll was destroyed, as for example in *I. horridus* where a specimen representing a circlet of sporophylls still attached to an axis has been interpreted by Brown (1962, Pl. 9 Fig. 1,3) as being a "corm with sporangia tipped by ligules". The *Isoetites* scales with a small apical tip seem more likely to represent entire sporophylls rather than the basal part of fertile leaves, and their small apical tip more likely represents their actual extremity and not the ligule. Moreover, the fact that these *Isoetites* sporophylls with short pointed or blunt apices are often found associated with long, apparently sterile, leaves and are still closely packed (see Brown, 1958 Fig. 3; Hickey, 1977; Collinson, 1991, Fig. 7h; Barale, 1999, Fig. 5)—as in the circlets of sporophylls of *I. horridus, A. zeilleri, Annalepis* sp. and *Lepacyclotes ellipticus* - suggests that they represented distinct organs, occupied a place distinct from that with sterile leaves, and became detached or abcised from an axis. *Isoetites daharensis* (Fig. 7b) is particularly interesting in this respect since it has a rounded rhizomorph with roots and a short stem bearing distal scale-like sporophylls and elongate sterile leaves (Barale, 1999). Such a plant does not conform to *Isoetes* (Fig. 7d) in which the sterile and fertile leaves are morphologically similar (Jermy, 1990) but more closely resembles *Annalepis* or *Pleuromeia* in which the fertile and sterile leaves differ and are located in distinct places on the plant. *Isoetites daharensis* (Fig. 6m) is all the more interesting because its sporophylls are of the *Annalepis*-type, as are those of *I. horridus* (Fig. 6n). However, contrary to the latter, in *I. daharensis* the leaves are not lacunate. In most of these fossil lycopsids, the length of the corm plus stem is unknown.

In *I. daharensis* (Fig. 7b), the rounded corm + stem are 11 cm tall (Barale, 1999) as in *Annalepis zeilleri* (Fig. 7a). In *Isoetites phyllophila* (Fig. 7c), the rhizomorph is elongate, conical, and 6 cm high (Skog et al., 1992). However, as noticed by Skog and Hill (1992), there are no broadly ranging studies on the shape of the stems at various stages in development of extant species of *Isoetes* stripped of their appendages and de-corticated, so it is difficult to compare fossil and living plants. In *Stylites* for example, the stem reaches 20 cm long (Rauh and Falk, 1959). Likewise, there are no broadly ranging studies on the distribution of the fertile and sterile leaves in extant *Isoetes*.

Clearly, most of the *Isoetites* specimens need further investigation and better illustrations, all the more since this genus is regarded as representing primitive *Isoetes* and is included in the subgenus *Euphyllum,* together with three extant species of *Isoetes* (Hickey, 1986, 1990; Taylor and Hickey, 1992).

Nevertheless, this comparative analysis of the sporophylls of *Annalepis* and related taxa (*Tomiostrobus, Lepacyclotes, Skilliostrobus,* some *Isoetites*) demonstrates that all these sporophylls have a similar basal unit which is narrow at the base, widens distally and extends by a short, roughly triangular distal portion ending with a more or less long pointed apical tip. In all there is a long oval adaxial sporangium and a ligule immediately distal to it. In most of them moreover, the proximal portion appears to bear lateral wings which are restricted to the area below the widened portion of the leaf. The sporophyll of *Isoetites horridus* figured by Hickey (1977, Pl. 1 figs. 6,7) is especially interesting in this respect because it shows particularly well the distal widening (Fig. 6n), suggesting that initially it also had lateral wings restricted to the proximal part.

RESEMBLANCES WITH *ISOETES* AND LEPIDODENDRID SPOROPHYLLS.—Besides the ultrastructural similarities of the spores of *Annalepis* and *Isoetes* (Lugardon et al. 2000b; Lugardon and Grauvogel-Stamm, in prep.), there are still some other morphological resemblances between both genera which deserve to be noticed.

Indeed, some *Annalepis* sporophylls (Fig. 8c) closely resemble the developing leaf primordia of *Isoetes* (Fig. 8e), which have a short pointed apex devoid of air chambers and an almost fully developed ligule, proximal to this apex (Kubitzki and Borchert, 1964). Such a resemblance shows how an *Annalepis* sporophyll may have developed into an *Isoetes* sporophyll. It would be instructive to know the shape and growth sequence of the wings in young *Isoetes* leaves but this development has not been studied (J. Hickey, personal information, September 1999).

Likewise it is noteworthy that the *Annalepis* sporophylls much resemble the scale leaves which occur in many *Isoetes* species and differ greatly from the typical *Isoetes* long leafy sporophylls. These scale leaves which are mostly unknown to *Isoetes* non-specialists are present at the growing apex and at the base of the corm, but are also said to occur below the spirally arranged leafy sporophylls, sometimes intermingled with them, and to form two or three whorls in old corms (Duthie, 1929; Stolze and Hickey, 1983; Hickey, 1986; Srivastava and Wagai, 1996). Thus for example, within the range of variation

of *Annalepis* sp. there are sporophylls (Fig. 8c) which are very similar to the scale leaves of *Isoetes montezumae* A.A. Eaton (Stolze and Hickey, 1983, Fig. 10c) or *I. coromandelina* Lin. fils (Srivastava and Wagai, 1996, Pl. 4 Fig. 1) (Fig. 8d). Likewise, the sporangium bearing scales with a small pointed apical tip (Fig. 6o) which occur in *Isoetites choffati* (Teixeira, 1948) among the long leafy *Isoetes*-like sporophylls, and which resemble the sporophylls of *Annalepis,* might also represent such scale leaves. According to Hickey (pers. com.), the scale leaves of *Isoetes* are environmentally induced and occur in distinct zones (alternating with typical fertile leaves) as a result of seasonality. Also according to Hickey (1986), they have a protective function for the corm and represent leaf primordia in which the development has become arrested. However, their resemblance with *Annalepis* sporophylls suggests that they also might have a phylogenetical significance and might represent vestigial remnants of ancestral sporophylls, indicating that the genus *Annalepis* is an ancestor of *Isoetes.*

The sporophylls which have been described recently from the Triassic of South China and which have been assigned to several new *Annalepis* species, e.g. *A. angusta* (Fig. 6c), *A. sangzhiensis* (Fig. 6d), *A.brevicystis* (Fig. 6e) and *A. zeilleri* (sensu Meng 1998) are also particularly interesting in the comparison between *Annalepis* and *Isoetes.* Indeed, these sporophylls which are characterized by a long, narrow (spur-like) outgrowth, arising distal to the ligular pit, running across the short, wide, triangular to rounded distal portion and extending far beyond it, also closely resemble the scale leaves which occur in *Isoetes* and which are characterized by a spine-like apex. In *I. mahadevensis* for instance, the scale apices are said to appear as spur-like outgrowths and to emerge abruptly from an almost rounded region (Srivastava and Wagai, 1996). In *Annalepis zeilleri* (sensu Meng 1998) moreover, this apical outgrowth is described as containing transverse partitions. Meng (1998) interpreted it as the leaf tip of the sporophylls. According to Hickey (pers. com.), it is comparable to the "subula" of the *Isoetes* leaves, i.e. their distal awl-like shaped outgrowth. Meng (1998, 2000) also noticed the similarity of the *Annalepis* sporophylls with *Isoetes* leaves, and suggested that *A. brevicystis,* especially, might be ancestral to the living genus.

The new Chinese *Annalepis* species are moreover particularly interesting because they allow to understand some unexplained features of *Tomiostrobus radiatus* (Fig. 6h). Indeed, the two parallel lines shown to arise distal to the ligule and to extend apparently out from the fragmentary apex of the sporophylls in the rough drawings of Dobruskina (1985, Fig. 1b-e, g, i, n), more likely represent partially preserved apical spine-like outgrowths comparable to those described by Meng (1995, 1998, 2000) in several Chinese *Annalepis* species and compared by Hickey (pers. com.) to the subula of the *Isoetes* leaves. In a sporophyll of *T. radiatus* figured by Yaroshenko (1988, Fig. 11), the long apex appears moreover to contain transverse ridges in the middle, though not noticed by the author, which might represent collapsed air chambers. However, none of the fragmentary sporophylls attributed by Sadovnikov (1982) to several distinct *Tomiostrobus* species (Fig. 6i) seems to contain such

partitions. Likewise, in none of the specimens of *Tomiostrobus* from the Lower Triassic of East and North Siberia (Russia) which one of us (L. G.-S.) could observe, the apex shows such features. Comparable unexplained spur-like outgrowths seem to exist in *Annalepis* sp. and *A. zeilleri* but they are badly preserved and have not yet been described (Grauvogel-Stamm and Lugardon, in preparation; Grauvogel-Stamm and Kelber, in preparation).

However, in spite of these resemblances there are some differences in the shape of the fertile area and lateral wings between the *Annalepis* sporophylls and the typical *Isoetes* long fertile leaves. In *Isoetes* the proximal portion is wide at the base, narrows distally and extends upwards in a long leafy extremity (Fig. 8j), whereas in *Annalepis* and allied genera the proximal portion is narrow at the base, widens distally and extends by a short more or less triangular portion ending in a small pointed apical tip (Fig. 8a, b). In *Annalepis* moreover the lateral wings are restricted to the area below the distal widening whereas in *Isoetes* the winged borders, now commonly termed alae, extend upwards over a variable length (Kott and Britton, 1985). It is worthy of note that in most of the extant *Isoetes* species, these alae are also restricted to the proximal portion of the leaf (Hickey, 1986; Taylor and Hickey, 1992) but, in contrast to *Annalepis,* they are not limited by a distal widening of that proximal portion.

In fact the shape of the proximal part and lateral wings of *Annalepis* sporophylls more resembles that of the basal unit of the sporophylls of the Carboniferous lepidodendrids *Lepidostrobus* Brongniart, *Lepidostrobopsis* Abbott and *Lepidocarpopsis* Abbott, as already noticed by Grauvogel-Stamm and Duringer (1983, Figs. 3a, b). These lepidodendrid sporophylls that have usually a long, wide, upturned leafy distal portion termed lamina in most descriptions, also have a basal unit consisting of a proximal portion bearing an adaxial sporangium and sometimes lateral wings, which is termed pedicel (Fig. 8f-i). It is noteworthy that the proximal portion of these sporophylls widens distally as in *Annalepis* or *Isoetites horridus* (Hickey, 1977) and that the wings, when present, are restricted to the area below the distal widening of the proximal portion. The sporophyll of *Lepidophyllum jenneyi* White (1899, Pl. 59 Fig. 2) which shows very clearly the distally widened proximal portion was regarded as having lost its lateral wings while it was interpreted by Abbott (1963) as having a T-shaped pedicel and was transferred by this author to the genus *Lepidostrobus* (Fig. 8h). In *Lepidocarpopsis lanceolatus* (Lindley and Hutton) Abbott on the contrary, the pedicel is clearly alate, exceeding the width of the sporangium (Fig. 8i). The sporophylls of *Cyclostigma kiltorkense* Haughton (Fig. 8f) from the Upper Devonian of Ireland (Chaloner, 1968) and *Lepidostrobus bohdarowiczii* Bochenski (1936) from the Carboniferous of Poland (Fig. 8g) also have such a T-shaped pedicel and a long distal lamina, but their pedicel seems to be devoid of lateral wings. However, according to Taylor and Hickey (1992), the alation which characterizes the sporophylls of the arborescent lepidodendrids and those of the isoetaleans is a plesiomorphy and therefore is not phylogenetically informative.

The terminology in the lycopsid reproductive organs

Comparisons of the *Annalepis* sporophylls with those of the arborescent lycopsids and those of *Isoetes* underscore a problem of terminology. Indeed, as there is no standardization for the different groups of lycopsids, various terminologies occurred which make rather complex and confusing the comparative studies. As regards to the lepidodendrids, the proximal and distal portions of the sporophylls are respectively termed "pedicel" and "lamina" by most of the authors (Abbott, 1963; Chaloner, 1967; Emberger, 1968; Bateman et al. 1992; Stewart and Rothwell, 1993). Emberger (1968, p.179) noticed that the proximal portion is equivalent to a petiole bearing a sporangium. In contrast, Taylor and Taylor (1993, p.270) defined the lepidodendrid sporophylls (*Lepidocarpon* Scott) as consisting of two lateral laminae and a distal extension. The similarities between the *Annalepis* sporophylls and those of the lepidodendrids led Grauvogel-Stamm and Duringer (1983) to use for *Annalepis* the terminology applied to the latter by most authors. However the resemblances between *Annalepis* and *Isoetes* emphasized for the first time in this study, required to change the terminology employed previously, as it has been done above.

In contrast to the lepidodendrids, in *Isoetes* the proximal portion of the sporophyll is termed "vagina" while its distal portion is called "subula". The current use in the terminology of *Isoetes* of these Latin words has been introduced by Hickey (1985) whereas, previously, they were employed only in the Latin diagnosis (Braun, 1868; Duthie, 1929; Wanntorp, 1970). However the use of these Latin words complements those which were already in use for *Isoetes*, such as "ala", "ligula", "velum" and "labium". As for the lateral wings (or alae) which are present in the proximal portion of the *Isoetes* leaves, they are regarded as the restricted remnants of the lamina (Hickey, pers. com.). In fact the meaning of these Latin words describes the features of the different portions of the leafy sporophylls. Thus, the portion containing the alae is called "vagina" which means "sheath", because the wings are slightly enveloping, as shown for example in the drawings of Wanntorp (1970). On the other hand, as the distal outgrowth of the *Isoetes* sporophylls is awl-like shaped, it has been called "subula" which means "awl".

The terminology such as applied to *Isoetes* seems to be applicable to some of the Chinese species of *Annalepis* (Meng 1995, 1998, 2000), as noticed by Hickey (pers. com. 1999) who recognized a subula in some of them, but it does not seem applicable to all the *Annalepis*-like sporophylls, particularly those in which there is no subula. However, the leaf primordium of *Isoetes* also seems to be devoid of a subula. Thus, only the shape and growth sequence of the wings and the subula in young *Isoetes* leaves would allow to establish the exact homologies with *Annalepis* but this development has not yet been studied (Hickey, pers. com., September 1999).

Conclusions

The comparative analysis of the Triassic lycopsids *Pleuromeia* and *Annalepis* using new macromorphological and ultrastructural data provides signif-

icant support for their close relationship with each other and with the living genus *Isoetes* and more ancient lycopsids, some of which extend back to the Devonian.

A close affinity between *Pleuromeia* and *Annalepis* appears unquestionable. Preliminary observations of some rather well preserved specimens of *Annalepis* indicate that they have furrowed, bilaterally symmetrical rhizomorphs as in *Pleuromeia*. The sporophylls, with their adaxial sporangium and distal ligule, are comparable in both genera, except that the sporophylls of *Annalepis* are wedge-shaped and have well-marked membranous wings which are seemingly missing in *Pleuromeia*. Moreover, although they are monolete, the microspores of *Annalepis* clearly show the same ultrastructural features as the trilete microspores of *Pleuromeia,* i.e. a thin exospore with a thinner, nonfolded apertural area combined with laminated zones, and a thicker spongy outer wall largely dissociated from the exospore and divided into two protruding lips above the aperture.

Most of the features shared by *Pleuromeia* and *Annalepis* also appear very similar to those of the living *Isoetes*. The comparative study of the rhizomorphs of *Pleuromeia* and *Isoetes* demonstrates that these organs have many points of structural and developmental correspondence. The ultrastructural features of the exospore and the spongy outer wall in the *Pleuromeia* and *Annalepis* microspores are remarkably similar and homologous with the walls of the *Isoetes* microspores (the latter having in addition a third, superficial wall, the perispore). This similarity between fossil and recent microspores is all the more striking because the fine structure of the *Isoetes* microspores differs markedly from that of all the other extant pteridophyte spores, including those of *Selaginella*. Indeed, despite some ultrastructural resemblances reflecting the relationships between *Isoetes* and *Selaginella,* the microspores of the latter show a number of unambiguous differences which strongly suggest that *Isoetes* is much more closely related to *Pleuromeia* and *Annalepis* than it is to *Selaginella*. The resemblances in morphology of the sporophylls also deserve to be noted. Snigirevskaya (1989) stressed the similarity between the sporophylls of *Pleuromeia* and the basal fertile region of *Isoetes* leaves. However, the clearly winged sporophylls of *Annalepis* show a stronger resemblance to *Isoetes*. In fact, the most important difference lies in the growth habit of the Triassic genera and that of *Isoetes*. Like some lycopsids from the Carboniferous (*Chaloneria cormosa*) and the Devonian (*Clevelandodendron ohioensis*), the Triassic lycopsids *Pleuromeia* and *Annalepis* have an elongated stem covered with entirely sterile leaves and a terminal, well-defined cone with short sporophylls, whereas the living genus consists of a short corm with long lacunate leaves, most of which bear a basal sporangium. However these differences do not seem to be very significant since they are rather common in the lycopsids where they are regarded as simple variations among related genera. The fact that in *Isoetes* many or all the leaves are fertile can be explained either by the loss of sterile leaves (Pigg, 2001) or, more likely, by the evolutionary reduction and transfer of the reproductive function to the vegetative leaves, as in the Gymnosperms and the Angiosperms (Nozeran, 1955).

All the similarities shown by both Triassic genera and *Isoetes* surely indicate close connections between them. Nevertheless, at first glance, they are not clearly indicative of a sister-group or an ancestor-descendant relationship and do not reveal the exact role played by *Pleuromeia* and *Annalepis* in the evolutionary history of *Isoetes*. However, there are some further noteworthy resemblances between *Annalepis* and *Isoetes* which strongly suggest that *Annalepis* is closer to the extant genus than *Pleuromeia* and that it is ancestral to *Isoetes*. Recently Meng (1998, 2000) also made this assumption. Besides their winged sporophylls/leaves noted above, there is, indeed, a striking similarity between the *Annalepis* sporophylls and the leaf primordia and scale leaves of the extant genus. Especially the scale-leaves which occur in many *Isoetes* species, and appreciably resemble *Annalepis* sporophylls, might be vestigial structures and might represent remnants of ancestral sporophylls, which might indicate that originally *Isoetes* had distinct sterile and fertile leaves as in *Pleuromeia* and *Annalepis*. The microspores of *Annalepis* are monolete as in *Isoetes*, whereas they are trilete in *Pleuromeia*. Moreover, the similarities between *Annalepis* and some Cretaceous and Tertiary fossils attributed to the genus *Isoetites* give further support to an ancestor-descendant relationship between *Annalepis* and *Isoetes*. The newly described species *Isoetites daharensis* from the Lower Cretaceous of Tunisia (Barale 1999), which is the best known among these plants, shows quite clearly that its sporophylls are remarkably similar to those of *Annalepis* and differ morphologically from the sterile leaves. Furthermore, its rhizomorph is cormose as in the Triassic genus, although not bilaterally symmetrical. As discussed above, *Isoetites horridus* from the Tertiary of North America (Brown 1939, 1958; Hickey 1977; Collinson 1991) was probably organized similarly, bearing its *Annalepis*-type sporophylls and sterile lacunate leaves in distinct areas, unlike *Isoetes*. These similarities strongly suggest that *Isoetites* and *Annalepis* are closely related and that *Isoetites* may have evolved from *Annalepis*. Thus the Triassic genus *Annalepis* seems to have played a more direct role in the evolutionary history of *Isoetes* than *Pleuromeia*. As regards to *Isoetites daharensis* and *I. horridus*, they may represent a group of lycopsids intermediate between *Annalepis* and *Isoetes*, and may indicate which structural changes occurred during this evolution. Thus the evolutionary series '*Annalepis—Isoetites—Isoetes*' appears very likely. Unfortunately, most of the *Isoetites* species are poorly known. They are usually identified on the sole basis of their lacunate leaves whereas the most important features, such as the precise shape and structure of the fertile parts and the microspore ultrastructure, remain unknown. The fine structure of *Minerisporites mirabilis*, the megaspore of *I. horridus* (Collinson, 1991), shows rather clearly the features of the isoetalean megaspores, but it does not provide any more precise informations on the affinities of *Isoetites*. Further and more thorough investigations of all the *Isoetites* material would be essential for definitely establishing the evolutionary series from the Triassic lycopsids to *Isoetes*. Besides, the fact that *Annalepis* and *Pleuromeia* are contemporaneous suggests that an ancestor-descendant relationship cannot exist between them. Therefore it is inferred that *Pleuromeia* either became extinct, or

originated within the nonalate species of *Isoetes*. However the latter hypothesis seems unlikely in view of the apparent homogeneity of the living genus which strongly suggests a monophyletic origin for it.

Some of the typical features of *Pleuromeia* and *Annalepis* are also present in more ancient lycopsids, providing some evidence of their ancestral affinities. The bilateral symmetry of the rhizomorph is known from the Devonian (Pigg, 2001). The sporophylls of the Carboniferous lepidodendrids, especially those with lateral wings, and those of more ancient lycopsids such as *Cyclostigma kiltorkense* from the Devonian of Ireland (Chaloner, 1968) are comparable in some respects to *Annalepis*. The special ultrastructural features of the microspores shared by the Triassic *Pleuromeia* and *Annalepis*, as well as by the extant *Isoetes*, also occur in *Polysporia* (Lugardon et al., 2000), *Chaloneria*, and other Carboniferous lycopsids. Moreover, it is of note that the trilete microspores of *Bisporangiostrobus harrisii* Chitaley and McGregor (1988) from the Upper Devonian of Pennsylvania contain proximal papillae as do the microspores of *Chaloneria/Polysporia, Annalepis, Pleuromeia* and *Isoetes*. This is all the more interesting since *Cyclostigma kiltorkense*, which has cones similar to those of *B. harrisii* (Stewart and Rothwell, 1993), also has a bilobed, bilaterally symmetrical rhizomorph as in *Pleuromeia, Annalepis* and *Isoetes*. Moreover several Frasnian-Famennian (Late Devonian) dispersed spores have ultrastructural characteristics comparable to those of the microspores of the Triassic genera and the extant genus (unpublished observations, B.L.). Thus it appears that the main features characterizing the rhizomorph and the microspores of living *Isoetes* appeared very long ago and remained outstandingly unchanged from the Devonian to the present. It is likely that these features characterize a particular group among the rhizomorphic lycopsids and that this group probably has a very remote origin. However, this group cannot be defined at the present time, notably because there are no accurate data on the microspore ultrastructure of most of the fossil lycopsids, mainly the arborescent lepidodendraleans. Methodical fine studies of in situ microspores of these lycopsids would surely supply significant information on the origin, relationships and delimitation of the different groups of isoetalean lycopsids.

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Diversification and Relationships of Extant Homosporous Lycopods

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ABSTRACT.—A series of phylogenetic analyses using nucleotide sequence data have resolved many aspects of the relationships in a group of land plants that until recently had received comparatively little attention, the homosporous lycopsids (Lycopodiaceae). The family includes no more than 400–500 living species but the group has evolved as an isolated lineage since the Early Devonian (390 Mya). Despite this ancient history, patterns emerging through the phylogenetic analyses imply that most diversification in this group is comparatively recent. The Lower Jurassic stem section *Lycoxylon indicum* indicates a minimum age for the split between *Lycopodium* and *Lycopodiella* at 208 Mya, and reticulate fossil spores from the Early Jurassic indicate that early cladogenesis in *Lycopodium* is of equivalent age. In the diverse predominantly epiphytic *Huperzia* group, biogeographic data indicates that 85–90 % of all living species result from cladogenic events postdating the final rifting of S. America and Africa in Mid to Late Cretaceous. The timing of these events coincides with the radiation of Angiosperms, and the diversification of epiphytic *Huperzia* was likely mediated by the development of broad leaved Angiosperm rain forests. Results indicate a single origin of epiphytism in *Huperzia*, but there have been at least two reversals to the terrestrial habit in the neotropics. The diversification of a large secondarily terrestrial clade, including about 80 montane high altitude species, was likely triggered by the Andean orogeny in the Mid Miocene, no more than 15 Mya.

Lycopsids are an ancient group with an extensive fossil record, and small herbaceous species are among the most easily recognizable and conspicuous elements of the early land flora. Because of their lengthy fossil record, the group was soon recognized as the product of an early cladogenic event in land plant evolution (Banks, 1968, 1975; Bower, 1908). Recent phylogenetic analyses support this view and indicate that lycopsids are sister to all remaining vascular plants (Doyle, 1998; Duff and Nickrent, 1999; Kenrick and Crane, 1997; Kranz and Huss, 1996; Raubeson and Jansen, 1992). The fossil record provides strong evidence that the three extant lineages (Lycopodiaceae, Selaginellaceae and Isoetaceae) also originated in the Palaeozoic. The occurrence of Protolepidodendrales in the Early Devonian (Kenrick and Crane, 1997) suggests a minimum age of 390 Myr for the split between Lycopodiaceae and the ligulate clade including Selaginellaceae and Isoetaceae. Furthermore, the occurrence of Isoetales in the Late Devonian (Kenrick and Crane, 1997) suggests a minimum age of 377 Myr for the split between extant Isoetaceae and Selaginellaceae. Despite the antiquity of these events, little is known about the diversification of the crown groups. Although the fossil record includes taxa showing similarities to extant Lycopodiaceae (Skog and Hill, 1992; Thomas, 1992), Selaginellaceae (Thomas, 1992, 1997) and Isoetaceae (Pigg, 1992; Retallack, 1997), their specific relationships to living species remain unclear.

One of the major problems has been the lack of well developed phylogenetic

hypotheses. Without such hypotheses, there is no way of telling whether the fossils are nested within the crown groups, or are part of the stem lineages. *Isoetes bestonii* Retallack for example has been identified as far back as the Triassic (Retallack, 1997). If shown to be part of the crown group of living *Isoetes,* this would certainly contribute to our understanding of the patterns of diversification of living species.

Separated from the ligulate lycopsids since the Early Devonian, the homosporous lycopods (Lycopodiaceae) have evolved as an independent lineage for at least 390 Myr. Morphologically, living species show striking similarities to some of the oldest vascular plants (Heuber, 1992; Kenrick and Crane, 1997), but from a palaeobotanical perspective Lycopodiaceae have been particularly problematic (Skog and Hill, 1992; Thomas, 1992). The macrofossil record is relatively meager and palaeobotanical work is hampered by recognition problems. Absence of clear morphological synapomorphies for the family makes assignments of fossil taxa difficult, and this is often based on absence of the characteristics of more distinctive groups such as Selaginellaceae. Another problem involves the superficial similarities between Lycopodiaceae and organ systems of other plant groups. These problems notwithstanding, Skog and Hill (1992) hypothesized that the major living groups within Lycopodiaceae were established during the Late Jurassic-Cretaceous. In other words, despite its apparent relict status, modern species diversity in Lycopodiaceae evolved comparatively recently and in parallel with that in more diverse, ecologically prominent groups such as flowering plants.

Over the last couple of years, I and my coauthors have been developing a phylogenetic hypothesis for living species of Lycopodiaceae (Wikström and Kenrick, 1997, 2000a, 2000b; Wikström *et al.*, 1999). Using molecular sequence data from the plastid *rbc*L gene, and from the *trn*L-*trn*F intron and spacer regions, we have conducted a number of phylogenetic analyses at different levels of the hierarchy in the family. These analyses provide empirical support for the idea that most cladogenesis in this ancient family is comparatively recent. Here I review some of the progress that has been made in our understanding of Lycopodiaceae relationships and the implications for interpreting the evolution of the family.

Relationships

Lycopodiaceae monophyly and *Phylloglossum.*—Recent cladistic analyses have shown that monophyly of Lycopodiaceae is at best weakly supported by morphological characters (Crane, 1990; Kenrick and Crane, 1997). Other studies have failed to identify unequivocal family synapomorphies (Wagner and Beitel, 1992), or indicate that Lycopodiaceae may be paraphyletic to a heterosporous lycopsid clade comprising Selaginellaceae, Isoetaceae and extinct relatives (Bateman, 1992). Molecular characters, on the other hand, furnish very strong support for monophyly of the family (Wikström and Kenrick, 1997, 2000a). Their *rbc*L sequence data unequivocally support Lycopodiaceae monophyly. Furthermore, molecular data resolve a basal split in Lycopodiaceae sep-

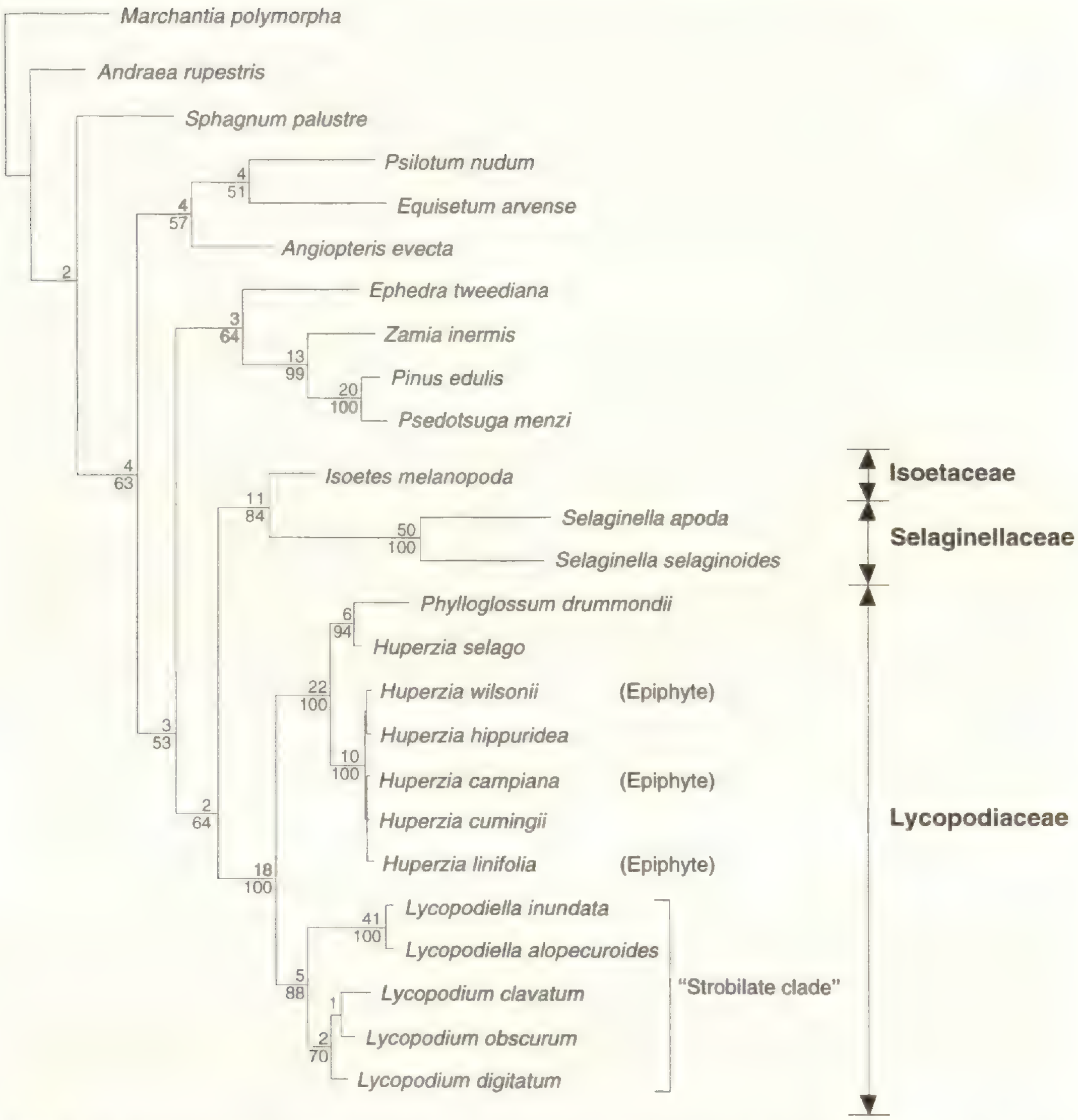

FIG. 1. A single most parsimonious tree indicating relationships of Lycopodiaceae. The tree is reproduced from the analyses of Wikström & Kenrick (1997) of *rbc*L sequence data. The tree is printed as a phylogram with branch lengths proportional to the amount of change along each branch and branch support is indicated by decay indices (above) and bootstrap values (below) for each branch.

arating a "strobilate clade", including *Lycopodium* L. and *Lycopodiella* Holub, from a *Huperzia* Bernh.—*Phylloglossum* Kunze clade. The single most parsimonious tree from Wikström and Kenrick (1997) is reproduced in figure 1 and shows branch support as measured by decay indices (Bremer, 1988; Donoghue *et al.*, 1992) and bootstrap values (Felsenstein, 1985). The support for monophyly of Lycopodiaceae is not entirely unexpected. Perhaps more surprising is the close relationship between *Phylloglossum* and *Huperzia* (Fig. 1). *Phylloglossum* is a monotypic genus occurring only in Australia and New Zealand,

and its highly divergent morphology has made its relationships to other species in Lycopodiaceae difficult to understand. Previous taxonomic treatments have either implicitly or explicitly classified it *incertae sedis* with respect to other groups in the family (Holub, 1985; Øllgaard, 1987; Wagner and Beitel, 1992). A close relationship between *Phylloglossum* and *Huperzia* is however consistent with a number of morphological features such as spore morphology (Breckon and Falk, 1974; Tryon and Lugardon, 1991), sporangial epidermis morphology (Øllgaard, 1975), phytochemistry (Markham *et al.*, 1983) and chromosome number (Blackwood, 1953). Other characters, and in particular the morphology of the perenniating tuber in *Phylloglossum*, led workers such as Bower (1935), Bruce (1976a) and Hackney (1950) to consider a close relationship between *Phylloglossum* and *Lycopodiella*. The *rbc*L sequence data, however, lend unequivocal support for the *Huperzia-Phylloglossum* relationship.

The evolution of *Phylloglossum* from either a *Huperzia* or a *Lycopodium-Lycopodiella* like ancestor implies a remarkable architectural transformation. This include the development of a new organ (perenniating tuber), a substantial reduction of the microphyll-bearing stem system, and the development of a unique stem anatomy. It is noteworthy though that the aspects of morphology likely to be the least affected by this overall architectural change, such as spore and sporangial epidermis morphology and phytochemistry, are also the most consistent with the *rbc*L data in supporting a *Huperzia* relationship.

THE "STROBILATE CLADE".—The "strobilate clade" includes the two genera *Lycopodium* and *Lycopodiella* (sensu Øllgaard, 1987), and although comprising no more than about 40 species each, 11 genera (Holub 1964, 1975, 1983, 1985, 1991) or 13 sections (Øllgaard 1987) have been recognized. My usage of generic and subgeneric names follows that of Øllgaard (1987). One factor contributing to the inflation of subgeneric groups is the segregation of several divergent species as monotypic groups. However, this segregation also reflects a genuine morphological pattern where subgeneric groups are very distinct, each including very similar species, but where there has been considerably more difficult to find similarities between such groups. Works looking at various morphological features such as stem anatomy (Bierhorst, 1971; Jones, 1905; Ogura, 1972), spore morphology (Tryon and Lugardon, 1991; Wilce, 1972), distribution of mucilage canals in trophophylls and sporophylls (Bruce, 1976b), branching pattern (Øllgaard, 1979), chromosome numbers (Wagner, 1992), sporangium epidermis morphology (Øllgaard, 1975), and gametophyte morphology (Bruce, 1976c) have tended to reinforce this pattern, and Øllgaard (1990) hypothesized that the subgeneric groups represent ancient evolutionary lineages.

Despite the success of recent taxonomic work in recognizing subgeneric groups and our increased knowledge about the morphological variation in these plants, there have been few explicit attempts to investigate the phylogenetic relationships of *Lycopodium* and *Lycopodiella*. Wagner and Beitel (1992) analyzed the relationships of N. American species and Wikström and Kenrick (1997) included representatives of both genera in their analyses. Both

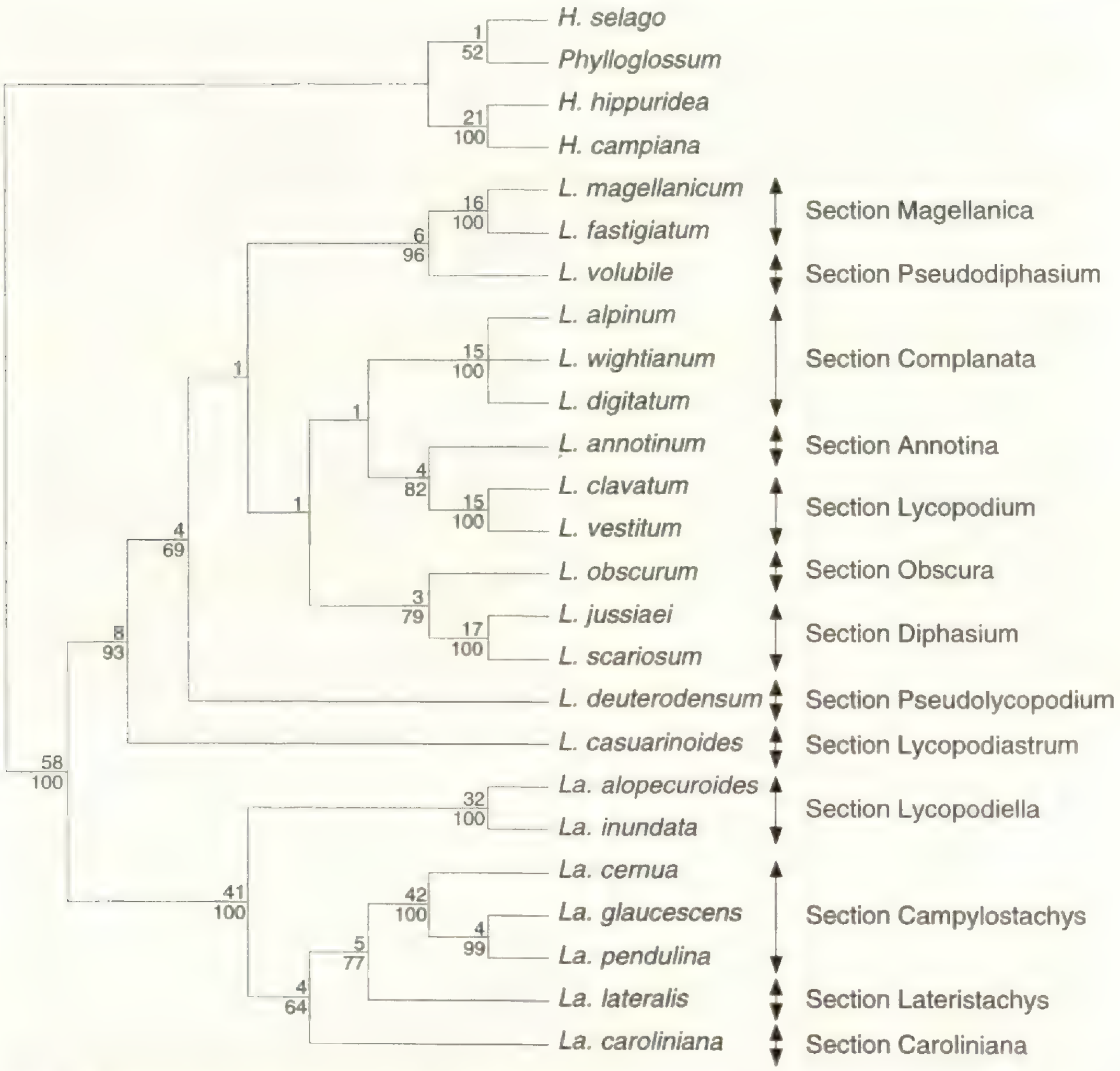

FIG. 2. Strict consensus of two most parsimonious trees indicating relationships within the "strobilate clade". The tree is reproduced from the analyses of Wikström and Kenrick (2000b) of combined *rbc*L gene and *trn*L intron sequence data. Branch support is indicated by decay indices (above) and bootstrap values (below) for each branch. Subgeneric groups (sections sensu Øllgaard, 1987) are shown to the right.

studies however, only included a limited number of species and the majority of the subgeneric groups recognized were not included. To address the relationships of *Lycopodium* and *Lycopodiella*, and specifically the relationships among subgeneric groups, we recently undertook an analysis using combined *rbc*L gene and *trn*L intron sequence data from a more extensive sample of taxa (Wikström and Kenrick, 2000b). The strict consensus tree from this analysis is reproduced in figure 2 showing branch support (decay indices and bootstrap values), and subgeneric groups (sections *sensu* Øllgaard, 1987) to the right. As seen in figure 2, the analyses support monophyly of both *Lycopodium* and *Lycopodiella* as well as monophyly of the sections. Branch support is however relatively low for a number of groups and some of the questions concerning

relationships among subgeneric groups remain unresolved. There are however some interesting patterns that not only provide an opportunity to calibrate the tree against the fossil record (see below) but that are supported by morphological data.

Lycopodium casuarinoides Spring. (section Lycopodiastrum) is grouped as sister to all remaining *Lycopodium*. This basal split in *Lycopodium* is supported by at least two morphological features: the lack of spore muri (Tryon and Lugardon, 1991; Wilce, 1972) and the presence of thick, lignified and sinuate sporangium cell walls in *L. casuarinoides* (Øllgaard, 1975). Both features are reminiscent of *Huperzia* and *Phylloglossum* and are best explained as retained plesiomorphic features. Another well supported grouping is the Pseudodiphasium (*Lycopodium volubile* G. Forster)—Magellanica clade. Even though there are few similarities in habit between *L. volubile* and section Magellanica, features of spore morphology (Wilce, 1972) and the absence of basal mucilage canals in their sporophylls (Bruce, 1976b; Øllgaard, 1987) support this grouping. In *Lycopodium,* two more subgeneric group relationships are supported. Section Obscura groups with section Diphasium and section Annotina groups with section Lycopodium.

Relationships within *Lycopodiella* are more difficult to resolve. The analyses of Wikström and Kenrick (2000b) included datasets from both the plastid *rbc*L gene and the plastid *trn*L intron regions. Results from the separate analyses however differed with respect to subgeneric group relationships and the support obtained in the combined analyses was moderate. The results are also somewhat surprising, considering morphological features. Section Caroliniana for example has several features, including isovalvate sporangia (Øllgaard, 1987) and absence of leaf veinal mucilage canals (Bruce, 1976b), that are best explained as retained plesiomorphic conditions. This would suggest a basal split in *Lycopodiella* separating section Caroliniana from remaining species, but such a relationship is not supported by molecular data (Wikström and Kenrick, 2000b).

Huperzia-Phylloglossum clade.—The *Huperzia-Phylloglossum* clade is by far more species diverse than the "strobilate clade", and *Huperzia* alone includes an estimated 300–400 species. The majority of these occur either as epiphytes in low to mid altitude montane rain forests of South America, Africa and South-East Asia, or as ground dwellers in open habitats of the upper montane neotropical rain forests and high altitude alpine vegetation of the Andean mountains.

Until recently, there had been no phylogenetic treatments of the group, and most taxonomic treatments document difficulties identifying discrete morphological features indicating infrageneric relationships. Øllgaard (1987) for example considered formal taxonomic decisions to be premature and considered his *H. selago* (L.) C. Martins and Schrank group to be the only reasonably distinct infrageneric entity. He did however recognize 22 informal species groups and an additional 10 subgroups (Øllgaard, 1987). Some of these are pantropical, and their distributions imply either ancient vicariance events,

possibly linked to the rifting of Pangea, or more recent transoceanic dispersal. He also recognized several different epiphytic and terrestrial groups, but it has been unclear how these are related to each other and whether epiphytism evolved once or iteratively.

To address these questions, we recently developed a phylogenetic hypothesis of *Huperzia* based on plastid *trn*L-*trn*F intron and spacer sequences (Wikström *et al.*, 1999). The analyses included 46 *Huperzia* species, representing 18 of Øllgaard's 22 informal species groups and most of the geographical regions where *Huperzia* species are found. The resulting strict consensus tree from these analyses is reproduced in figure 3 showing branch support (decay indices and bootstrap values), species groups (sensu Øllgaard, 1987), growth habit (terrestrial/epiphytic) as well as the occurrence of neotropical and paleotropical clades. The results indicate that many of the species groups recognized by Øllgaard (1987) are either polyphyletic (e.g., *H. phlegmaria* (L.) Rothm., *H. taxifolia* (Sw.) Trevisan and *H. verticillata* (L.f.) Trevisan groups), paraphyletic (e.g., *H. saururus* (Lam.) Trevisan group) or poorly supported (e.g., *H. reflexa* (Lam.) Trevisan group). The results however strongly support the existence of other interesting patterns. Epiphytism appears to have originated once within *Huperzia* with at least two independent reversals to a terrestrial habit (*H. hippuridea* (Christ) Holub and the clade including *H. saururus, H. brevifolia* (Grev. & Hook.) Holub and *H. reflexa* groups), both in the neotropics. With two exceptions (*H. ophioglossoides* (Lam.) Rothm. and *H. funiformis* (Spring) Trevisan), all the epiphytic and secondarily terrestrial species are split into a neotropical and a paleotropical clade. Within the neotropical clade, the terrestrial species in *H. saururus, H. brevifolia* and *H. reflexa* groups constitute a large, secondarily terrestrial clade. These patterns are all well supported by the *trn*L-*trn*F data and the existence of neotropical and paleotropical clades has also been corroborated by an extended *rbc*L analysis which included both neotropical and paleotropical epiphytes (Wikström and Kenrick, 2000a).

Comparing these results with respect to morphological data is difficult. There are few morphological features that can be used to define infrageneric groups, and characteristics that have been used are endpoints in morphological continua, resulting in arbitrary decisions on character state delimitations. Notwithstanding these problems, some of the patterns obtained in the molecular analyses are unexpected. For example, neotropical and paleotropical members of the *H. phlegmaria* and of the *H. verticillata* groups show conspicuous morphological similarities but, based on the molecular analyses, these morphological similarities are the result of convergent evolution. Furthermore, as homosporous plants they have usually been considered to disperse easily, but the molecular results indicate the existence of very strong biogeographic patterns within *Huperzia.* These patterns could perhaps be related to the posession of subterranean holosaprophytic gametophytes and aspects of their establishment. Similar gametophytes are however also found within *Lycopodium*, a genus that does not show corresponding patterns (Wikström and Kenrick, 2000b). Our current knowledge on aspects of the establishment and dynamics

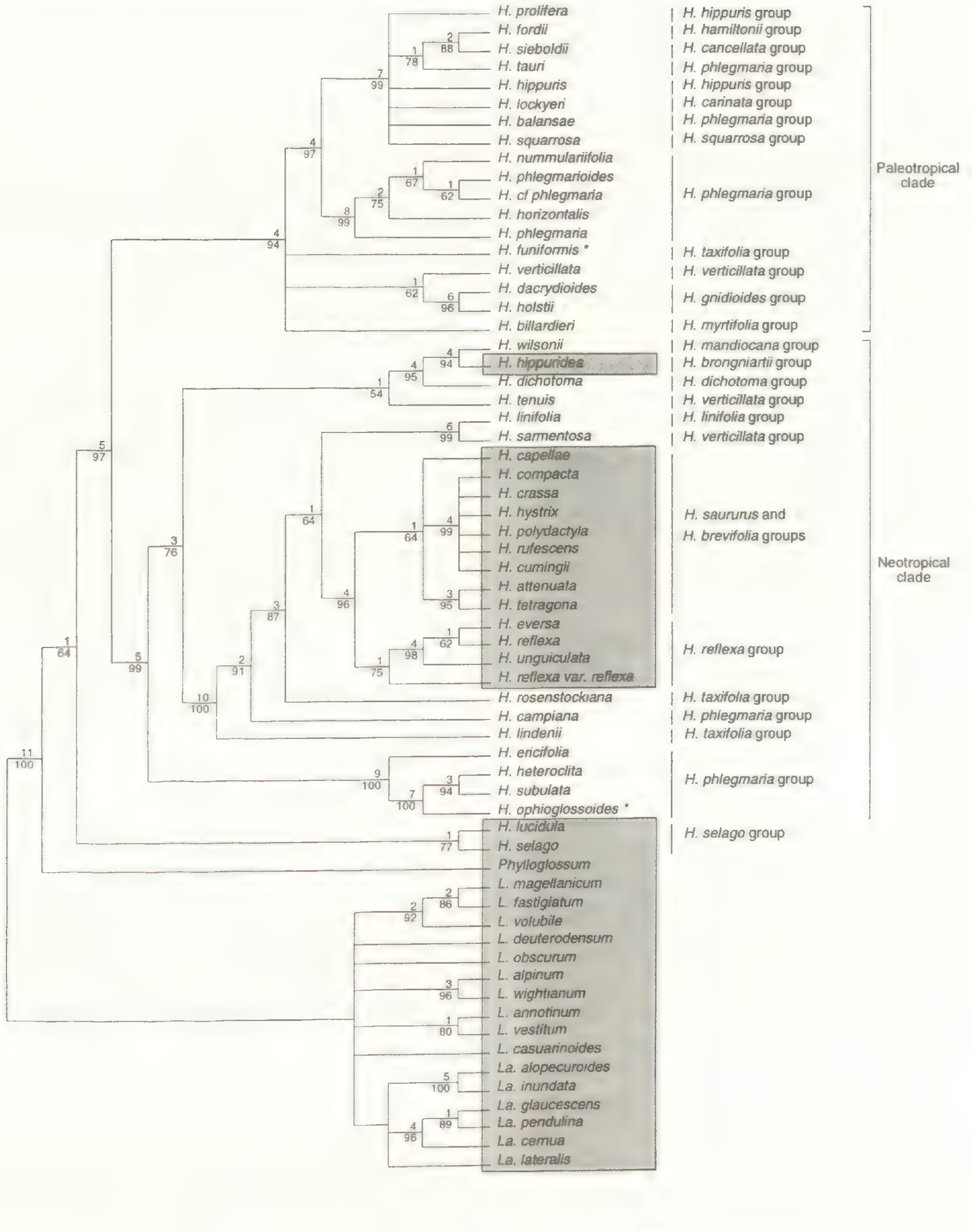

FIG. 3. Strict consensus of 16,924 most parsimonious trees indicating relationships within the *Huperzia-Phylloglossum* clade. The tree is reproduced from the analyses of Wikström *et al.* (1999) of *trn*L-*trn*F intron and spacer sequence data. Branch support is indicated by decay indices (above) and bootstrap values (below) for each branch. Informal species groups (sensu Øllgaard, 1987) are indicated to the right. The partition of epiphytic *Huperzia* into a neotropical and a paleotropical clade is also indicated. *H. funiformis* and *H. ophioglossoides* (marked with *) are exceptions to this clear biogeographic pattern. Taxa within grayed boxes are terrestrial and remaining species are epiphytic.

of lycopod populations is however limited, and before such knowledge is acquired, one can only speculate.

Patterns of Diversification

Calibration of the phylogenetic tree is critical to reconstructing the historical patterns and two approaches have been adopted. Where possible, information from the fossil record has been used. This includes the use of fragmentary plant remains, isolated organs and spores. The calibrations discussed here are however tentative and primarily based on a literature survey. As seen in figure 4, some of the calibrations based on fossil information indicate contradictory ages and further palaeobotanical work on reassembling whole plants is required. Ultimately, the fossils should of course not only be used for calibrations, but should also be included in the analyses. Attempts to correlate biogeographic data on living species with other geological data such as major tectonic events have also been done.

The macrofossil record of *Lycopodium* and *Lycopodiella* is meager (Skog and Hill, 1992). The middle Jurassic (Bajocian-Bathonian; 174 Mya) *Lycopodites falcatus* Lindley & Hutton is one of the better known species and its morphology was documented in detail by Harris (1961). He suggested an affinity with section Complanata, based on its flattened branch system with large lateral leaves and smaller dorsal and ventral leaves. If correct, this would suggest a minimum age of 174 Mya for the split between section Complanata and the clade including sections Lycopodium and Annotina (Fig. 4). Although the arguments for its lycopodiaceous affinity are convincing, critical morphological features such as stem anatomy and spore morphology are unknown and its position along the stem lineage of section Complanata as indicated in figure 4, is highly uncertain.

The mature stem anatomy of *Lycopodium* is highly distinctive with the xylem arranged in parallel bands (plectostele). This feature is characteristic of all living species. Srivastava (1946) documented what he considered to be a lycopodiaceous plectostele (*Lycoxylon indicum* Srivastava) from the Jurassic (Lias) of India (Fig. 5). Although completely decorticated, the arrangement of the xylem strands is highly reminiscent of living *Lycopodium*. This fossil evidence suggest a minimum age for the split between *Lycopodium* and *Lycopodiella* of 208 Mya (Fig. 4). The specimen of *Lycoxylon indicum* occurs in the same series of beds as *Lycopodites gracilis* Brogniart, a compression fossil that closely resembles *Lycopodites falcatus* (Skog and Hill, 1992). To determine if the two names represent different organ names for the same species would certainly contribute to our understanding of lycopod evolution.

Using fossil evidence for calibrating the tree is associated with a number of problems and potential sources of error (Doyle and Donoghue, 1993). First of all, synapomorphies shared by living taxa must be observable and recognizable in fossil taxa. Secondly, fossils only provide estimates of minimum ages and not maximum ones. If the chance of observing a particular feature is low due to a relatively meager fossil record, the minimum age implied by some rare

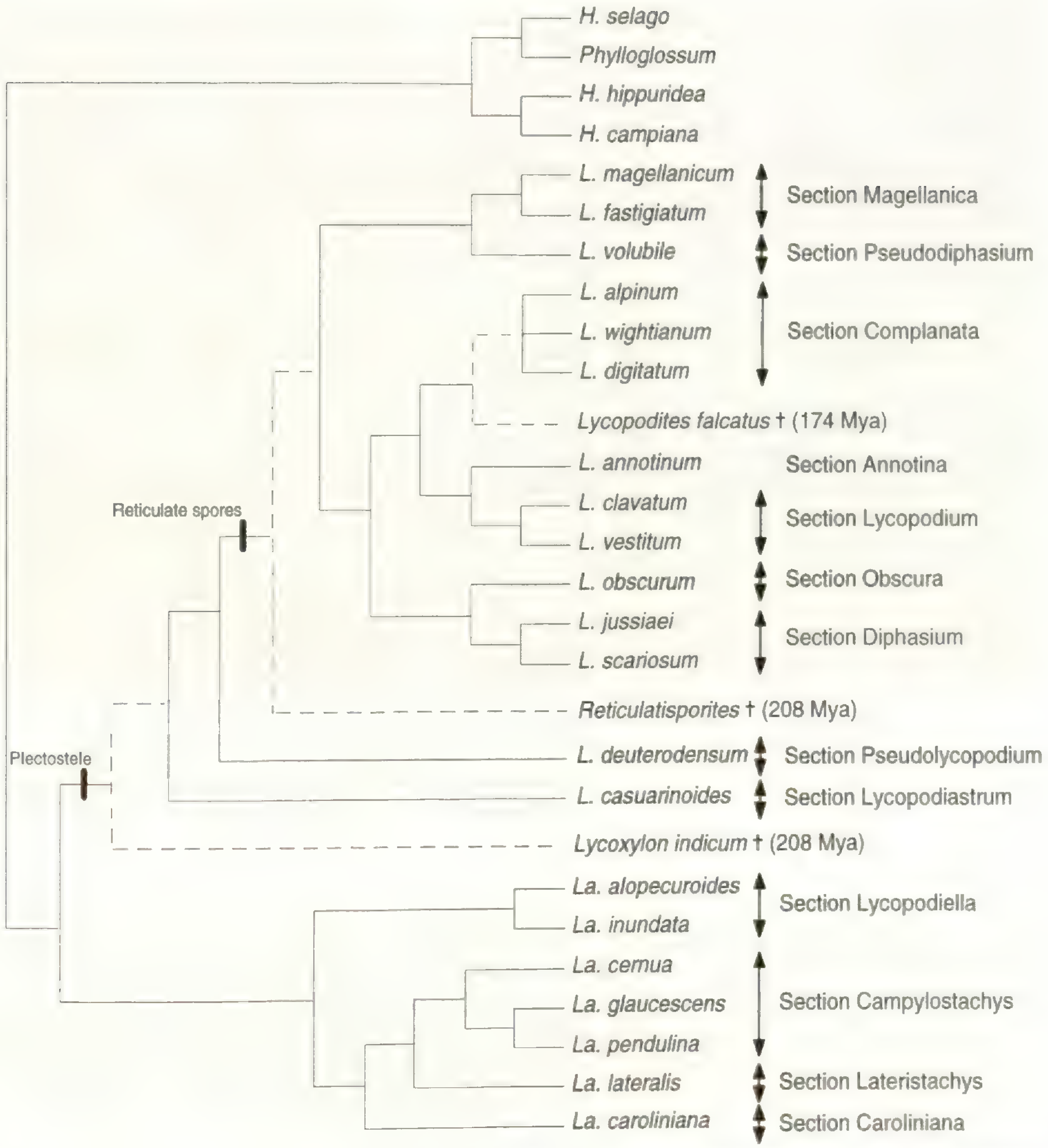

FIG. 4. Strict consensus of two most parsimonious trees from Wikström and Kenrick (2000b) including the fossils used for calibrating the tree. The fossils were not part of the analyses and have simply been inserted at the basalmost position implied by their characteristics. Ultimately the fossils should of course be included in the analyses, but our current knowledge of these fossils makes such an inclusion unfeasible at this stage.

observation of this feature can greatly underestimate the true age of its origin. Our confidence in a minimum age estimate based on fossil information therefore depends on the probability that failure of finding the feature in earlier geological records represents a "true absence" meaning it had not yet originated. In this respect, the most useful information for calibrating the ages of groups within the strobilate clade comes from the fossil spore record.

The record of fossil spores with putative *Lycopodium* affinities is quite ex-

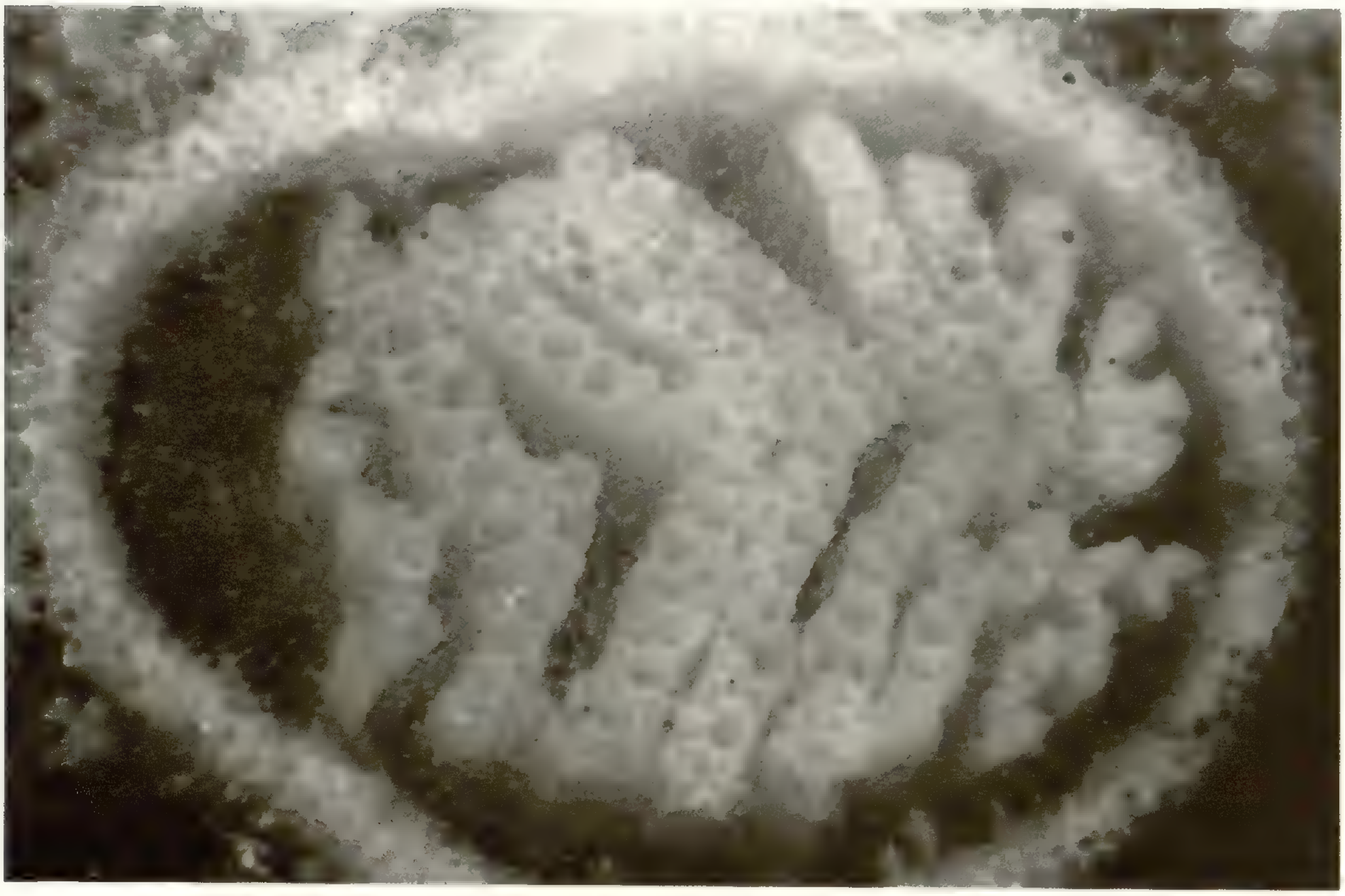

FIG. 5. *Lycoxylon indicum* (Birbal Sahni Institute of Palaeobotany, Lucknow: specimen K11/8). The fossil species was originally described by Srivastava (1946) from the Jurassic (Lias) of India. A parallel arrangement of the xylem, as seen in *Lycoxylon*, is characteristic of all living *Lycopodium* and this fossil evidence indicates a minimum age for the split between *Lycopodium* and *Lycopodiella* of 208 Mya (Fig. 4).

tensive, and there are detailed surveys of external spore morphology in living species documenting conspicuous features observable in fossil spores (Tryon and Lugardon, 1991; Wilce, 1972). Wilce (1972) recognized five different spore types within the family, four of which are found in the "strobilate clade". The rugulate type is found throughout *Lycopodiella* and the reticulate, scabrate, and bacculate types are found within *Lycopodium*. The reticulate type is typical of most species of *Lycopodium*. All but two of the living species, *L. casuarinoides* (scabrate spores) and *L. deuterodensum* Herter (bacculate spores) have this type and on the molecular phylogeny, the reticulate spore type appears as a unique synapomorphy within *Lycopodium* (Fig. 4). Fossil spores with a reticulate spore ornamentation resembling living *Lycopodium* have been documented under a number of different names such as *Retitriletes* Van der Hammen ex Pierce emend. Döring, Krutzsch, Mai & Schulz (Dettmann, 1986), *Neoraistrickia* Potonié, *Reticulatisporites* (Ibrahim) Potonié & Kremp, *Microreticulatisporites* (Knox) Potonié & Kremp, *Sestrosporites* Dettmann, *Coronatispora* Dettmann (Couper, 1953; Dettmann, 1986; Knox, 1950), *Lycopodiumsporites* Thiergart ex Delcourt & Sprumont and *Assamiasporites* Mehrotra & Sah (Kar and Mandal, 1984). Some of the *Retitriletes* species (Early Jurassic-Tertiary) are convincingly of *Lycopodium* affinity (Dettmann, 1986), and confirms the presence of *Lycopodium* by the Early Jurassic. Bharadwaj (1962) re-

ported *Lycopodiumsporites* from Upper Permian coal fields of India. Although less convincing, it indicates that early cladogenesis in *Lycopodium* may be older. Some of the reticulate fossil spores, assumed to have affinities with *Lycopodium,* will undoubtedly not survive a more critical evaluation. Others however might, and a synthesis of published data on the stratigraphic and geographic distributions of Lycopodiaceae spore fossils, and an evaluation targeted at identifying putative homologies among fossil spores and spores from living species, would clearly contribute to our understanding of the origin and diversification of *Lycopodium* and *Lycopodiella.*

About 85–90% of all living *Huperzia* species (all species groups except the *H. selago* group) are included in a tropical epiphytic clade, and the molecular results indicate that the origin of epiphytism predates a split into neotropical and paleotropical clades (Fig. 3). This biogeographic pattern is best explained by vicariance, specifically the rifting of S. America and Africa in Mid to Late Cretaceous (80–90 Mya). Biological exchange between S. America and Africa was becoming rare by this time (Taylor, 1991; Taylor, 1995; Windley, 1995) and it is therefore probable that most cladogenesis within these clades postdates the Mid Cretaceous. This conclusion is further corroborated by analyses using sequence divergence data. Results from analyses of sequence divergences using non parametric rate smoothing (Sanderson, 1997) resolve the diversification of the two clades as Late Cretaceous (Wikström & Kenrick, 2001). This implies that most modern species diversity originated in parallel with that of angiosperms (Crane, 1987). The timing and pattern of this diversification is likely related to ecological changes associated with the development of forest vegetation dominated by arborescent angiosperms.

The origin of a second monophyletic group within *Huperzia* correlates well with geological data. The clade including *H. saururus, H. brevifolia* and *H. reflexa* groups (Fig. 3) comprises an estimated 80 species and all are more or less restricted to high altitude habitats in the Andes mountains. Species of the *Huperzia reflexa* group occur in open habitats and pioneer vegetation in the transition zone between montane rain forests and the higher altitude alpine vegetation. Species of the *Huperzia saururus* and *H. brevifolia* groups include frost tolerant species and are almost exclusively found in high altitude alpine vegetation, and Øllgaard (1992) hypothesized that the *H. saururus* group originated as a response to the Andean orogeny. Results from the phylogenetic analyses confirms and extends this idea to include the *H. brevifolia* and *H. reflexa* groups. The diversification of this large, secondarily terrestrial clade seems to have been triggered by the development of open, alpine vegetation resulting from mountain building during the Andean orogeny. Mountains were probably well developed in the central Andes by the Mid Miocene (15 Mya) and in the northern Andes by the Miocene-Pliocene boundary (5 Mya; Taylor, 1995; Windley, 1995). Conservative estimates would place the origin of this secondarily terrestrial clade at about 15 Mya.

Conclusions

Available evidence indicates that most cladogenesis within the crown groups of living species of Lycopodiaceae is comparatively recent. Fossil evi-

dence provides a minimum age for basal cladogenesis in *Lycopodium* at Early Jurassic (208 Mya), and biogeographic evidence indicates that most cladogenesis within the *Huperzia-Phylloglossum* clade is even younger, postdating the Late Cretaceous (80 Mya). Considering the ancient split between homosporous and heterosporous lycopsids (Kenrick and Crane, 1997), this is somewhat surprising. The absence of family level synapomorphies however constitutes a major problem for calibrating the origin of the crown group of living Lycopodiaceae. Given our best estimate of phylogeny and our current knowledge of comparative morphology, there are no morphological features unequivocally originating along the stem lineage of extant Lycopodiaceae. Although the fossil record of Palaeozoic herbaceous lycopsids is rather fragmentary (Thomas, 1992), identifying such features would provide valuable information and could potentially provide the means for such a calibration.

The indication that the diversification of epiphytic *Huperzia* occurred in parallel with that of angiosperms, and may have been triggered and mediated by the development of angiosperm dominated rain forests may represent a more general pattern in land plants. Vitt (1984) and Buck *et al.* (2000) for example suggested a similar pattern among mosses. They hypothesized that the evolution of pleurocarpy in mosses might have been an adaptation for continuous growth and an epiphytic habit that occurred relatively recently and simultaneously with the diversification of angiosperms. This implies that perhaps as much as 70% of extant species diversity in mosses is of equally recent origin. Calibrating the origin of pleurocarpy will however be difficult considering the available fossil information. One could also speculate that similar patterns could be found within extant fern groups. The transition from a plant community dominated by pteridophytes, conifers, cycadophytes and non angiosperm seed plants to one completely dominated by angiosperms in the Late Cretaceous clearly had an enormous impact with respect to the composition and structure of the plant community (Crane, 1987). No doubt this also lead to a decrease in species diversity within a number of lineages but, at a different level, the development of a fundamentally closed canopy vegetation may also have provided new niches into which other lineages evolved and diversified.

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The Utility of Nuclear ITS, a *LEAFY* Homolog Intron, and Chloroplast *atpB-rbcL* Spacer Region Data in Phylogenetic Analyses and Species Delimitation in *Isoëtes*

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ABSTRACT.—Despite its ancient origins, its worldwide distribution, and adaptation to diverse habitats, *Isoëtes* has a highly conserved morphology. This feature has made it difficult to resolve species and species relationships using morphological characters. In this paper, we report the utility of nucleotide sequences from the nuclear internal transcribed spacer (ITS) regions, chloroplast *atpB/rbcL* intergenic spacer region, and second intron of a *LEAFY* (*LFY*) homolog for identifying species relationships, delimiting basic diploid species, and determining hybrid origins. Variation in the ITS regions and *atpB/rbcL* spacer is most useful at the family level in *Isoëtes* and the *LFY* second intron is appropriate at the species and population level. The tree resulting from an analysis of the combined nuclear ITS and chloroplast *atpB/rbcL* spacer contains three major well supported clades (bootstrap ≥ 99%): an Old-World/California clade (*I. abyssinica, I. longissima, I. velata, I. nuttallii,* and *I. orcuttii*), an Asian/Australian clade (*I. taiwanensis, I. japonica, I. kirkii,* and *I. drummondii*), and a poorly resolved clade consisting of nine North American species. To further resolve and delimit the North American species, a combination of the *LEAFY* second intron and ITS data was used. The resulting consensus tree has limited resolution, supporting the hypothesis that the North American species complex radiated rapidly. The combination of *LFY* and ITS data provided numerous characters, both substitutions and indels, that are useful in species delimitation and identification of cryptic species. ITS sequence data, through additive banding and sequence misalignment, is also useful in confirming interspecific hybrids and determining their parental origins.

Isoëtes is a cosmopolitan genus of heterosporous lycopsids containing 200 or more species. Despite the probable ancient Paleozoic origins of *Isoëtes,* its worldwide distribution, and its diverse ecological adaptations (from seasonal ephemeral terrestrials to obligate evergreen aquatics), *Isoëtes* species are singularly lacking in morphological variation (Taylor and Hickey 1992). The taxonomic difficuties caused by conserved morphology are compounded by homoplasy and allopolyploid speciation. Because of these problems, *Isoëtes* is a prime candidate for additional sources of data, such as DNA sequences.

A typical *Isoëtes* sporophyte consists of a buried rootstock bearing a rosette of simple, linear sporophylls. Each sporophyll contains four longitudinal, septate canals and an ovoid sporangium near its base. Each sporangium contains either scores of globose megaspores or thousands of ellipsoid microspores. A flap of tissue, called the velum, partly or completely covers the sporangium. Above the sporangium is a triangular ligule.

Since the Paleozoic Era, there have been numerous opportunities for species

of *Isoëtes* to adapt repeatedly to terrestrial habitats during drier conditions and to aquatic habitats in wetter periods. The range of habitats in which the species are found today appears to be a reflection of these past adaptations. For example, some species are evergreen aquatics in glacial lakes. Other species are ephemeral, seasonal terrestrials in soil pockets on exposed rock outcrops. Still others are amphibious, found in and along intermittent streams. A few species invade old fields and roadside ditches. *Isoëtes* appears to be an opportunistic pioneer able to adapt to a wide range of habitats.

Repeated adaptations to aquatic and terrestrial environments over time could lead to reversals, parallelisms, and convergences in morphological data sets. Thus it is possible that homoplasious morphological character states have been used to identify and relate species. A phylogeny of *Isoëtes*, based on few, potentially homoplasious character states, would probably be an inaccurate reconstruction. Therefore, alternative data sets are critical for reconstructing phylogenies and defining species within *Isoëtes*.

Chromosome counts have provided some insight into the evolutionary history of *Isoëtes*. For example, about one-half of the North American species are basic diploids ($2n = 22$, $\times = 11$). The other half form a polyploid series of tetraploid ($2n = 44$), hexaploid ($2n = 66$), octaploid ($2n = 88$), and decaploid ($2n = 110$) species. These chromosome numbers indicate that *Isoëtes* has evolved in two ways. Basic diploid species have evolved by ecological isolation and genetic divergence as taxa adapted to terrestrial and aquatic habitats. Polyploid species have evolved through interspecific hybridization and chromosome doubling when divergent species occupied overlapping ranges. Interspecific hybrids, recognized by their irregularly formed and nonviable megaspores, are commonly encountered where two or more species occur together. At least fifteen different interspecific hybrids of *Isoëtes* have been detected among North American species (Taylor *et al.*, 1993; Montgomery and Taylor, 1994; Britton and Brunton, 1995, 1996; Musselman *et al.*, 1995, 1996, 1997). Interspecific hybrids and putative allopolyploids have caused confusion in defining species because they possess character states intermediate between the two parental species.

The main purpose of this paper is to show the usefulness of sequence data for ITS, the *atpB/rbcL* spacer region, and especially the second intron of the *LEAFY* (*LFY*) ortholog, to address phylogenetic questions. Future work will result in more extensive data (including many more species sampled) for resolving phylogenetic relationships and questions related to biogeography, species delimitation, and hybrid origins.

Variation in the ITS regions and *atpB/rbcL* spacer is most useful at the family level in *Isoëtes* and the *LFY* second intron is appropriate at the species and population level. The two ITS regions are located between the 18S and 26S nuclear ribosomal genes and separated by the 5.8S gene. There are multiple copies of this ribosomal array in the genome, but they appear to undergo rapid concerted evolution and all copies appear to be virtually identical in *Isoëtes* (Wendel *et al.*, 1995; Baldwin *et al.*, 1995; our data). The two ITS regions together are about 590 bp in length in *Isoëtes* but nucleotide substitutions and

insertions/deletions (indels) are common. ITS regions have been used extensively to study angiosperm taxa at the species and generic level (Baldwin *et al.*, 1995).

The *atpB/rbcL* intergenic spacer is located between two highly conserved chloroplast genes, *atpB* and *rbcL*. This spacer region is approximately 750 bp long, but indels are common. Spacer sequences have been used in many phylogenetic studies (e.g., Golenberg *et al.*, 1993; Manen *et al.*, 1994; Hoot and Douglas, 1998).

LFY is a meristem identity gene that encodes a transcription factor involved in the activation of homeotic genes which control flowering in *Arabidopsis* (Parcy *et al.*, 1998). *LFY* homologs have been identified in *Pinus, Picea, Welwitschia, Gnetum,* ferns, *Huperzia, Lycopodium,* and *Selaginella* (Frohlich and Meyerowitz, 1997; Mouradov *et al.*, 1998; Frohlich, pers. comm.; Therrien, pers. comm.). *LFY* is usually found in one copy in higher plants but *Isoëtes* has two copies. These two copies, including short stretches of the flanking exons, differ in length by about 100 bp (long copy ca. 1150 bp; short copy ca. 1050 bp). The partial exon sequences of the two copies are highly conserved and readily aligned, but the two introns have very different nucleotide sequences. For our work on *Isoëtes,* only the long copy of intron 2 was sequenced.

Materials and Methods

Total DNA was isolated from 0.3–2.0 g of fresh or silica-dried leaf tissue (Table 1) following the CTAB extraction procedure of Doyle & Doyle (1987).

The ITS regions, including the 5.8S gene, were amplified by the polymerase chain reaction using primers 1830F, located in the 18S gene, and 25R, located in the 26S gene (for primer sequences, see Nickrent *et al.*, 1994). Double-stranded amplifications in 100 μL reactions were conducted with the following reagents: 20 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$,10 M DMSO, 50 μM each dNTP, 0.25 μM of each amplification primer, and 2.5 U Taq polymerase. After overlaying each reaction with mineral oil, genomic DNA (210–500 ng) was added. Reaction conditions consisted of 40 cycles of 94° C for 30 sec, 45°C for 30 sec, and 72°C for 2 min for the denaturation, annealing, and extension steps, respectively. The first cycle was preceeded by a 4 min denaturation step and the last cycle by a 5 min extension step. The *atpB-rbcL* intergenic spacer region was amplified as described in Hoot and Douglas (1995) using primers located within the *atpB* and *rbcL* genes for both amplification and sequencing.

The second intron of *LFY* (long copy) was amplified using the degenerate primers LFsxl-3 and LFtxr (Frohlich and Meyerowitz, 1997). For five species, the two copies of *LFY* present in *Isoëtes* were substantially different in size and could be separated on a 1.5% agarose gel overnight. The two bands were then excised, reamplified using the melted gel plug as template, and used for sequencing. The resulting sequences were then aligned to locate a conserved region within the second intron that could be used to design internal ampli-

TABLE 1. Collection data for species analyzed.

Isoëtes	*Country/State*	*Collector*	*Collection*
I. abyssinica Chiov.	Kenya	Gastony	97–101
I. butleri Engelm.	USA/Kansas	McGregor	*s.n.*
I. coromandelina L.	India	Srivastave	*s.n.*
I. drummondii A. Br.	Australia	Hoot	*s.n.*
I. echinospora Dur.	USA/Maine	Taylor	5137
I. echinospora Dur.	USA/Montana	Taylor	*s.n.*
I. echinospora Dur.	USA/Wisconsin	Taylor	5097
I. engelmannii A. Br.	USA/Michigan	Taylor	*s.n.*
I. Engelmannii A. Br.	USA/Missouri	Taylor	*s.n.*
I. Engelmannii A. Br.	USA/Virginia	Taylor	6015
I. flaccida Shuttlew.	USA/Florida	Taylor	5214
I. hawaiiensis Taylor & Wagner	USA/Hawaii	Taylor	5658
I. japonica A. Br.	Japan	Takahashi	*s.n.*
I. kirkii A. Br.	New Zealand	Woodland	*s.n.*
I. lithophila Pfeiffer	USA/Texas	Taylor	4653
I. longissima Bory	Spain	Taylor	5409
I. melanopoda Gay & Dur.	USA/Louisiana	Lark	*s.n.*
I. melanopoda Gay & Dur.	USA/Mississippi	Leonard	*s.n.*
I. melanopoda Gay & Dur.	USA/Arkansas	Johnson	*s.n.*
I. melanospora Engelm.	USA/Georgia	Taylor	4849
I. nuttallii A. Br.	USA/California	Taylor	5446
I. orcuttii A. A. Eat.	USA/California	Taylor	5447
I. prototypus Britton	Canada	Brunton	12350
I. taiwanensis DeVol	Taiwan	Kuo	*s.n.*
I. tegetiformans Rury	USA/Georgia	Taylor	5182
I. valida (A.A. Eat.) Clute	USA/North Carolina	Taylor	*s.n.*
I. velata A. Br.	Spain	Prada	*s.n.*

fication primers. These internal primers were specific to just the long copy of the *LFY* intron and are located approximately half way through the intron. The long copy of the *LFY* second intron was then amplified in two parts. Amplification followed the same protocol described above for ITS except that an initial 25 μl amplification reaction was done with Ready-To-Go Beads (Amersham Pharmacia Biotech) and the resulting PCR product used as the template for future reactions.

Following amplification, double-stranded PCR product was freed of remaining nucleotides and primers by electrophoresis on a low melting 2% agarose gel. The bands were visualized with UV light source, removed as gel plugs, and melted at 70°C. DNA was further purified and concentrated using Wizard Columns (Promega), following the manufacturer's protocols. Purified PCR products were sequenced on an ABI 373-Stretch automated sequencer. The two amplification primers were used for each region with additional internal sequencing primers employed for the ITS and *atpB-rbcL* spacer regions (primer sequences available upon request from S. Hoot).

Alignment of DNA sequences was accomplished to a rough approximation using Sequencher 3.0 (Gene Codes Corp.) with subsequent manual corrections. The criteria for indel alignment and scoring are as described in Hoot and Doug-

las (1998). Phylogenetically informative indels (variable in two or more taxa) were scored as one event at the end of the data set.

Fitch parsimony of the separate and combined data sets were performed with PAUP* vers.4.0b2a (Swofford, 1998) using the branch and bound search option. To assess branch support, PAUP* was used to perform bootstrap analyses (Felsenstein, 1985) with 500 replications, the branch and bound search option, and maximum number of trees saved set at 2000.

Isoëtes is morphologically very distinct and does not appear to be closely related to any extant pteridophytes. We are currently working on rooting our molecular trees using various genera within the Lycopodiaceae, the nearest sister group to *Isoëtes* (Manhart, 1994; Wikström and Kenrick 1997). As this work is not yet complete, we chose *I. coromandelina* L. f. as the root of our tree based on three lines of evidence: 1) preliminary *rbcL* data for selected species of *Isoëtes* and lycopods, 2) previous morphological work suggesting that the more plesiomorphic character states and geographic distributions are found in section *Coromandelina* (Taylor & Hickey, 1992), and 3) molecular evolution in *Isoëtes* appears to evolve in a clocklike fashion, suggesting that the root of the tree will be among the species with the greatest number of substitutions (this work and unpublished preliminary data). For the analysis of the combined LFY/ITS data (North American species complex), the tree was rooted using the ITS sequence for *I. drummondii* A. Br., based on the results of our worldwide phylogeny (Fig. 1). Due to sequencing difficulties, a sequence for *I. drummondii* was not available for *LFY*; the data for that region is scored as missing for this species in the combined data set.

Results

Relationships of Basic Diploid Species.—A combination of ITS and *atpB-rbcL* spacer data was used to evaluate relationships on a global scale and resulted in seven most parsimonious trees based on 176 informative characters (312 variable characters) with a consistency index excluding uninformative characters (CI) = 0.79 and retention index (RI) = 0.90. One of the seven shortest trees is presented in Fig. 1.

Three major well supported clades (bootstrap ≥ 99%) are recognized: an Old-World/California clade (*I. nuttalii, I. orcuttii, I. abyssinica, I. longissima,* and *I. velata*), an Asian clade (*I. kirkii, I.drummondii, I. taiwanensis,* and *I. japonica*), and a North American clade (*I. flaccida, I. prototypus, I. melanopoda, I. lithophila, I. melanospora, I. engelmannii, I. valida, I. echinospora,* and *I. hawaiiensis*). The North American species are not monophyletic. *Isoëtes nuttallii* and *I. orcuttii* from California are nested within a clade consisting of Spanish and African species, separate from the remaining North American species. The North American clade, referred to in the future as the North American species complex, is poorly resolved, suggesting a possible rapid radiation event with subsequent speciation.

Species relationships and delimitation within the North American species complex.—To resolve and delimit species within the North American spe-

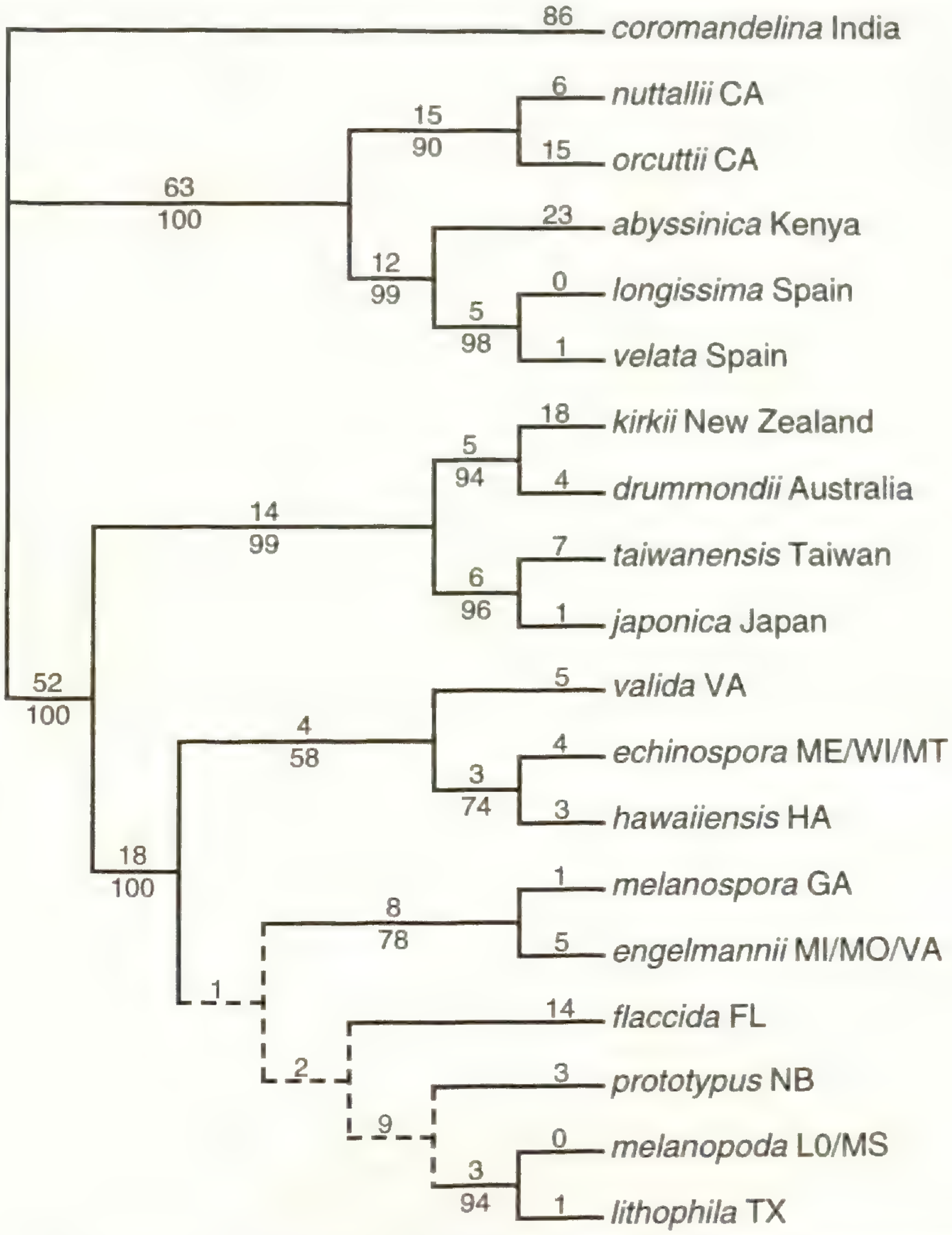

FIG. 1. One of the seven most parsimonious trees derived from the combined ITS and *atpB-rbcL* spacer region data. Numerals above the lines indicate the number of nucleotide changes supporting each branch (using accelerated transformation optimization); numbers below branches are bootstrap values. Dotted lines indicate branches that collapse in the strict consensus tree derived from multiple shortest trees.

cies complex, a combination of ITS and *LFY* intron data was used. The *LFY* intron data, consisting of 218 variable characters and 24 indels, is more than four times more variable than ITS (48 variable characters and four indels). The eight trees resulting from the analysis of *LFY* intron data alone (tree not presented) are largely similar to the combined *LFY*/ITS tree (Fig. 2), differing only in the position of *I. butleri.* (sister to *I. engelmannii* in the *LFY* tree). There was no resolution of the North American species complex using ITS data alone.

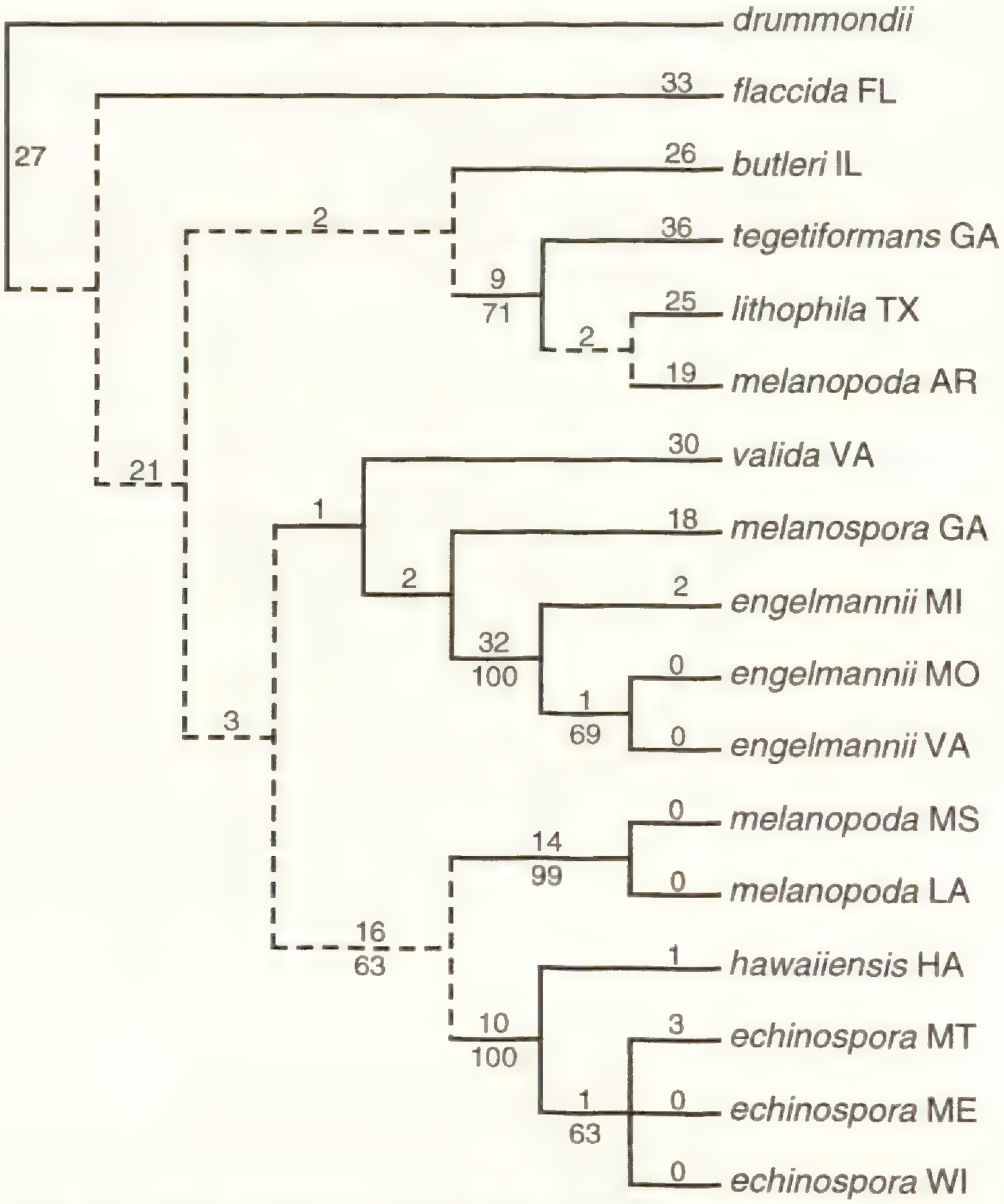

FIG. 2. One of six most parsimonious trees derived from the combined *LFY* intron 2 and ITS data. Numerals above the lines indicate the number of nucleotide changes supporting each branch (using accelerated transformation optimization); numbers below branches are bootstrap values. Dotted lines indicate branches that collapse in the strict consensus tree derived from multiple shortest trees.

One of the seven shortest trees (94 informative characters, CI = 0.77 and RI = 0.88) resulting from the analysis of the combined ITS and *LFY* intron data is presented in Fig. 2. The deeper branches of the tree collapse in the strict consensus tree, supporting the hypotheses that the North American species complex radiated rapidly.

Within the North American species complex, there is some resolution of species relationships (Fig. 2). *Isoëtes tegetiformans*, *I. lithophila*, and *I. melanopoda* (population from Arkansas) form a moderately well supported clade (9 substitutions, bootstrap = 71%). Unlike the results from a combination of ITS and spacer data, *I. melanopoda* populations from Louisiana and Mississippi

are weakly supported (bootstrap = 63%) as sister to a well supported clade consisting of *I. hawaiiensis* and *I. echinospora* (bootstrap = 100%). While the position of *I. valida* changes between the two trees derived from ITS/spacer and *LFY*/ITS, none of the relevant branches are well-supported (bootstrap ≤ 58%).

The combination of *LFY* and ITS data provide numerous characters, both substitutions and indels, which are useful in delimiting species (Fig. 2). Plants from three widely separated populations of *I. engelmannii* from Michigan, Missouri, and Virginia occur in the same well-supported clade (Fig. 2; 32 characters, bootstrap = 100%). Similarly, individuals from widely separated populations of *I. echinospora* from Maine, Wisconsin, and Montana, are nearly identical, only differing from each other by three unique sites found in the Montana population.

In contrast, the tree resulting from the combined *LFY* and ITS data indicates that *I. melanopoda* as currently defined is polyphyletic and composed of at least two species. *Isoëtes melanopoda* from Arkansas, differs from two populations of *I. melanopoda* from Louisiana and Mississippi by numerous substitution and indels. The former appears in a moderately-supported clade with *I. lithophila* and *I. tegetiformans* (bootstrap = 71%).

DETERMINING THE ORIGIN OF ALLOPOLYPLOID SPECIES.—We used nucleotide sequences of the ITS regions of nuclear ribosomal DNA to tentatively determine the origins of the interspecific hybrid *I.* ×*eatonii* Dodge and the allotetraploid *I. riparia* Engelm. ex A. Br. Taylor and Hickey (1992) provided data from distribution patterns, spore morphology and viability, electrophoretic profiles of leaf enzymes, and in vitro hybridization experiments supporting the hypothesis that the basic diploid species *I. echinospora* (2n = 22) and *I. engelmannii* (2n = 22) have crossed to form the sterile hybrid *I.* ×*eatonii* (2n = 22) and that chromosome doubling has produced the fertile allotetraploid, *I. riparia* (2n = 44).

When ITS sequences of *I. echinospora*, *I. engelmannii*, their hybrid, *I.* ×*eatonii*, and *I. riparia* are aligned and compared, nucleotide additivity and misalignment are apparent in the sequence chromatograms (Fig. 3). Nucleotide additivity occurs in *I.* ×*eatonii* and *I. riparia* at nucleotide positions where *I. echinospora* and *I. engelmannii* differ. Nucleotide misalignment occurs in *I.* ×*eatonii* and *I. riparia* in the 5′ to 3′ direction (determined by the direction of primer extension) where there is a nucleotide insertion or deletion in either *I. echinospor*a or *I. engelmannii* (Fig. 3). ITS nucleotide additivity occurs at each of 10 sites in *I.* ×*eatonii* and at 8 sites in *I. riparia* where *I. echinospora* and *I. engelmannii* differ (Table 2). There are also two indels in *I. echinospora* and *engelmannii*, leading to nucleotide misalignments (beginning at sites 49 and 548) in *I.* ×*eatonii* and *I. riparia*.

DISCUSSION

RELATIONSHIPS OF BASIC DIPLOID SPECIES.—The combination of nucleotide sequences from ITS and the *atpB/rbcL* intergenic spacer are effective in resolving

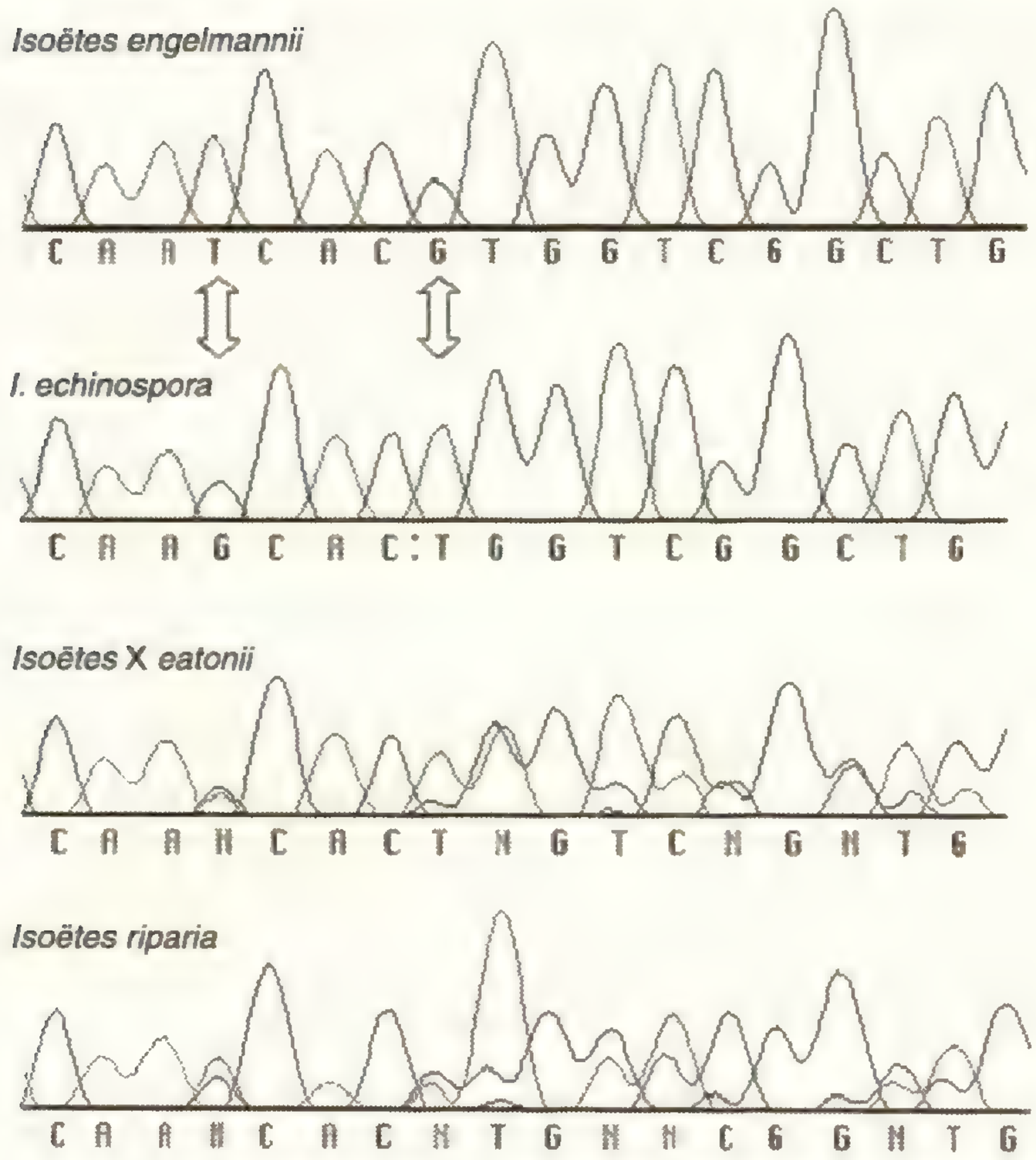
Isoëtes engelmannii
C A A T C A C G T G G T C G G C T G
I. echinospora
C A A G C A C T G G T C G G C T G
Isoëtes X eatonii
C A A N C A C T N G T C N G N T G
Isoëtes riparia
C A A N C A C N T G N N C G G N T G

FIG. 3. Aligned sequence chromatograms of *I. engelmannii*, *I. echinospora*, *I.* ×*eatonii*, and *I. riparia* for an 18 bp segment in the ITS2 region. The arrow on the left indicates site 544 where the two parental species, *I. engelmannii* and *I. echinospora*, differ in nucleotides and additive banding is found in the two taxa of hybrid origin, *I.* ×*eatonii*, and *I. riparia*. The arrow on the right indicates the location of a deletion in *I. echinospora* in relation to *I. engelmannii*. The two parental sequences found in *I.* ×*eatonii* and *I. riparia* are misaligned to the right of this deletion in the direction of the primer extension.

phylogenetic relationships of diploid species found throughout the world. These variable sequences and their rate of evolutionary change provide numerous characters (both nucleotide substitutions and indels) at this taxonomic level in *Isoëtes*.

The tree resulting from the combination of ITS and spacer data are of interest biogeographically (Fig. 1). The well supported Old World/California clade consists of species with Eurasian affinities. The inclusion of the two Californian species, *I. nutallii* and *I. orcuttii*, within this clade suggests that this group of *Isoëtes* may have previously had a distribution throughout Asia and into North America. *Isoëtes abyssinica* is sister to the two Spanish species, *I. longissima* and *I. velata*, indicating a strong affinity between at least some North African and European species. These results also support at least two origins for the North American species of *Isoëtes*, those with Eurasian affinities as described above and the many species found in the North American species complex. Current work is now underway which will greatly increase our sampling worldwide, further testing the above biogeographical hypotheses.

The lack of variation in the North American species complex using two

TABLE 2. ITS nucleotide additivity in *Isoëtes* × *eatonii* and *I. riparia* at sites where the parental species, *I. englemannii* and *I. echinospora*, differ.

	Sites and Nucleotides									
Taxon	39	83	274	314	319	321	544	627	644	679
I. engelmannii	A	C	T	G	G	A	T	C	G	G
I. echinospora	T	T	C	C	T	T	G	T	A	T
I. Xeatonii	A/T	C/T	C/T	C/G	G/T	A/T	G/T	C/T	A/G	G/T
I. riparia	T	C/T	C	C/G	G/T	A/T	G/T	C/T	A/G	G/T

different combined data sets, ITS/spacer (Fig. 1) and *LFY*/ITS (Fig. 2), suggests a rapid radiation in the North American species complex. However, the *LFY*/ITS tree does resolve a sister group relationship between *I. hawaiiensis* and three populations of *I. echinospora* (8 substitutions, 2 indels, bootstrap = 100%), suggesting that a founder population of *I. echinospora* made its way to Hawaii from the boreal regions of the Northern Hemisphere, possibly by means of migrating waterfowl. *Isoëtes hawaiiensis*, while exhibiting considerable morphological divergence from *I. echinospora*, is virtually identical in terms of molecular data (two character differences), suggesting that the event occurred relatively recently.

SPECIES DELIMITATION WITHIN THE NORTH AMERICAN SPECIES COMPLEX.—A persistent problem in molecular plant systematics has been the lack of DNA sequence regions variable enough to be used at the species and population level. The second *LFY* intron is proving invaluable in this regard. In *Isoëtes*, *LFY* is at least four times more variable than ITS, which is generally regarded as one of the more variable sequence regions available for systematic studies.

The species identity found in the *LFY*/ITS tree (Fig.2) is largely based on *LFY* data due to the greater variation found in this region. *LFY* provides numerous characters, both substitutions and indels, which can serve to delimit and identify species. For example, our results indicate that widely separated populations of *I. engelmannii* (Michigan, Missouri, and Virginia) and *I. echinospora* (Maine, Montana, and Wisconsin) are virtually identical.

In contrast, the *I. melanopoda* complex contains at least one cryptic species. The population of *I. melanopoda* from Arkansas is very different molecularly from the two populations from Louisiana and Mississippi, coming out within a moderately supported clade (bootstrap = 71%) consisting of *I. lithophila* from Texas and *I. tegetiformans* from Georgia. Work is currently underway to further test species limits for other *Isoëtes* taxa.

DETERMINING THE ORIGIN OF ALLOPOLYPLOID SPECIES.—Additive banding and sequence misalignment in ITS data from hybrids and allopolyploids are valuable tools in the confirmation of interspecific hybrids and the determination of their parental origins. For the most part, the additive banding found in the hybrid *I.* ×*eatonii* and the allotetraploid *I. riparia* confirm that their progenitors were *I. echinospora* and *I. engelmannii* (Table 2). Two of the sites (39, 274) do not show additivity in *I. riparia*, but do possess nucleotides at these

sites that are found in one of the putative parents. This loss of additivity may be due to the effects of concerted evolution, mutation events, or sequence differences in one of the diploid parents of *I. riparia* not detected with our sampling. We are currently studying hybrid origins of the above and other allopolyploids using *LFY* data. Because many more substitutions and indels occur with *LFY* data, cloning is being employed to separate the two parental copies. Our preliminary data for *LFY* also support the above findings for the origins of *I. riparia*.

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Enhancement of Fern Spore Germination and Gametophyte Growth in Artificial Media

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ABSTRACT.—Spores of bracken fern, *Pteridium aquilinum* were sown in medium with and without agar. No clear trend emerged for germination, but in 14 days, significantly more plants passed from the filamentous to two-dimensional state on agar-solidified medium than in liquid shake-flasks. Spores of *Pteridium aquilinum*, *Athyrium filix-femina*, *Dryopteris expansa* and *Anemia phyllitidis* were sown in media containing sucrose. Percentage germination of all four species was significantly enhanced by the inclusion of sucrose.

Fern gametophytes have been described as ideal experimental organisms for use in scientific studies and as model multicellular systems (e.g., Miller, 1968; Hickok *et al.*, 1987). One of the main attractions of these plants is their minimal nutritional requirements, and most researchers have exploited them in cultures employing artificial media. Agar-solidified medium has been the substrate used most routinely for the artificial culture of fern gametophytes, but disadvantages have been reported. Agar can be toxic to plants (e.g., Debergh, 1983), it can perturb sexual expression of ferns (Rubin & Paolillo, 1983), and can slow fern development (Dyer, 1979). Douglas & Sheffield (1992) showed dry weight yield of two species of fern gametophytes, *Pteridium aquilinum* and *Anemia phyllitidis*, to be significantly higher in liquid than in the same medium solidified with agar. This study did not establish, however, whether these higher yields reflected increased germination rates (therefore larger numbers of individuals), higher rates of growth of similar numbers of individuals, or both. Data in Douglas (1994) indicated differences in germination of *Pteridium* and *Anemia* between liquid and agar-solidified medium, with germination percentage significantly higher in liquid medium for both species. Subsequent work in our laboratory using spores from the same collections as used in those experiments, has not repeated these findings. Camloh (1993) found no significant differences in germination of the epiphytic fern *Platycerium bifurcatum* between liquid and agar medium, but reported that "development" was enhanced by liquid medium. The first aim of this investigation was therefore to establish whether liquid medium promotes germination and/or growth of one of the two species used in the study of Douglas and Sheffield, viz. *Pteridium aquilinum* (bracken fern).

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Many workers have included metabolisable sugars in the artificial medium used to germinate fern spores, but no clear trends have emerged from such studies. Hurel-Py (1955) reported that sugars inhibited germination of *Alsophila australis*. Camloh (1993) found that the inclusion of sucrose in liquid medium produced no significant inhibition or stimulation of germination of *P. bifurcatum* spores. Pilot experiments of Douglas (1994) suggested that sucrose greatly enhanced germination of *P. aquilinum* and *A. phyllitidis*. The second aim of this investigation was therefore to establish whether the addition of sucrose enhances or inhibits germination of fern spores. Four species were chosen from different families (*Pteridium aquilinum, Athyrium filix-femina, Dryopteris expansa* and *Anemia phyllitidis*), in an attempt to generate broadly applicable findings.

Materials and Methods

Spores of *Pteridium aquilinum, Athyrium filix-femina, Dryopteris expansa* and *Anemia phyllitidis* were collected and stored (dry in glass vials) at 4°C for either one *(Pteridium),* two (*Athyrium, Dryopteris*) or twelve years *(Anemia).* Spores of the first three taxa were harvested from natural populations of British ferns, and the *Anemia* spores were a kind donation from the glasshouse population of Professor H. Schraudolf (University of Ulm). All spores were sieved through a fine nylon mesh (45μm pore size, Lockertex) to separate them from sporangial debris. They were weighed and kept in plastic vials containing approximately 10ml distilled water with one drop of detergent (wetting agent) at room temperature for 18–24 h. Spores were then surface sterilised with 2.5% (vol/vol) aqueous sodium hypochlorite solution (10–14% available chlorine, BDH) for 2 min, then rinsed three times in sterile distilled water. They were subsequently suspended in sterile 0.5% (w/vol) high viscosity carboxymethyl cellulose (BDH) to allow even distribution of the spores in suspension for inoculation. The basic mineral medium used throughout was that of Moore (1903) adjusted to pH 6.25.

Agar vs Liquid Medium.—Medium containing 1.5gl ^{1} Sigma bacteriological grade agar was dispensed into petri dishes; liquid medium was dispensed as 150ml aliquots into 250ml conical flasks and the flasks sealed with a polyurethane foam bung. Both media were autoclaved at 15lb, 120°C for 15 min.

Liquid Medium With and Without Sucrose.—Medium without sucrose, and medium containing 0.05 and 0.2M sucrose (Sigma) was dispensed into flasks as above, and autoclaved at 10lb, 115°C for 10 min.

Inoculation.—Each spore suspension was adjusted to contain 150,000 (± 500) spores using a Sedgewick Rafter chamber and inoculum added aseptically to each dish or flask to give an initial spore concentration of ca. 1000 spores per ml of medium. The spores were evenly distributed over the agar surface using a flamed glass spreader and excess moisture allowed to evaporate in a laminar flow unit before sealing the dishes with Nescofilm. Flasks were placed on a

rotary shaker at 96rpm and dishes cultured on the bench in the same room, illuminated by daylight fluorescent tubes providing photosynthetically active radiation of 400–700 nm of 106 (±21) μEm $^{-2}$s $^{-1}$ light at the level of the culture surface on a 16h light 8h dark cycle, at 20 (±2)°C.

SAMPLING.—Cultures were sampled after 7 days' incubation in the above conditions for all treatments, and after 14 days for liquid vs agar treatments. Numbers of germinated and non-germinated spores were counted after 7d, and the numbers of individuals that had established 2D growth and those still filamentous after 14d were counted; 250 individuals were counted per replicate.

ANALYSES.—Individual replicates were subjected to analysis of variance, spore germination and 2D growth data were subjected to an independent samples t-test for equality of means, and the means of each different sucrose treatment subjected to chi-square tests to ascertain which sources of variation generated differences between treatments.

RESULTS

AGAR VS LIQUID MEDIUM.—Percentage germination was higher for *Pteridium* spores sown on agar than in liquid medium; the percentage of individuals that had established 2D growth by 14d of culture was considerably higher for both species in agar than in liquid medium (see Table 1). The treatment effects were confirmed when the experiment was repeated, although the overall percentage germination and the percentage variation was not the same for each repeat. Results obtained from spores sown in liquid medium were routinely far more variable than those obtained from agar-solidified cultures.

LIQUID MEDIUM WITH AND WITHOUT SUCROSE.—Percentage germination was very different for different species (see Tables 2–5) and "species tested" was the most highly significant factor accounting for differences between treatments ($F = 139.51$, $p<0.001$, see Table 6). Germination in different concentrations of sucrose was in the order of 0<0.05<0.2M for all species except *Anemia,* where germination was slightly less in 0.2 than in 0.05M, but both were higher than 0M (see Tables 2–5). The differences between treatments due to concentration of sucrose was significant ($F = 5.88$, $p<0.01$, see Table 6).

DISCUSSION

The results presented here for germination of spores of *Pteridium* sown in liquid vs agar-solidified medium do not mirror those obtained by Douglas (1994). The latter study reported 64(± 4)% germination in liquid, 42(± 4)% in agar, i.e. lower germination on agar than in liquid—the reverse of the findings reported herein. Douglas (1994) found less difference for *Anemia,* but the trend was the same. We could speculate upon an interaction between medium composition and spore age, as our spores of *Pteridium* were only one year old, and those of Douglas were two years old for *Pteridium* and seven years old for

TABLE 1. The mean percentage of *P. aquilinum* spores that germinated, and the mean percentage of individuals that had progressed to 2D growth after 14 d in liquid and agar-solidified Moore's medium (n = 4 for all, s.e. in parentheses).

	Agar	Liquid	P
Germination	85.2 (1.1)	60.0 (16.6)	0.026*
2D growth	94.8 (1.2)	34.2 (13.6)	0.011*

* Less than 0.05, therefore differences between treatments significant

TABLE 2. The mean percentage germination of spores sown in liquid medium containing different concentrations of sucrose for *P. aquilinum* (n = 4).

Sucrose concentration	% germination
0 M	30.4 (8.0)
0.05 M	39.3 (10.2)
0.2 M	43.3 (18.1)

Between concentrations chi-square 145.94 $P < 0.001$

TABLE 3. The mean percentage germination of spores sown in liquid medium containing different concentrations of sucrose for *A. phyllitidis* (n = 4).

Sucrose concentration	% germination
0 M	54.0 (3.1)
0.05 M	57.8 (8.6)
0.2 M	56.4 (8.6)

Between concentrations chi-square 7.54 $P < 0.05$

TABLE 4. The mean percentage germination of spores sown in liquid medium containing different concentrations of sucrose for *D. expansa* (n = 4 for 0M and 0.05M sucrose; n = 3 for 0.2M sucrose).

Sucrose concentration	% germination
0 M	57.8 (3.3)
0.05 M	61.1 (4.6)
0.2 M	66.0 (4.5)

Between concentrations chi-square 33.34 $P < 0.001$

TABLE 5. The mean percentage germination of spores sown in liquid medium containing different concentrations of sucrose for *Athyrium filix-femina* (n = 4 for 0M and 0.05M sucrose; n = 1 for 0.2M sucrose).

Sucrose concentration	% germination
0 M	87.2 (1.3)
0.05 M	91.2 (2.6)
0.2 M	94.7 (na)

Between concentrations chi-square 50.61 $P < 0.001$

TABLE 6. Chi-square tests of differences attributable to the variables examined.

Source of variation:	Chi-square	Probability
Species	4952.35	$P < 0.001$
Concentrations within species	237.45	$P < 0.001$

Anemia, and we obtained higher germination percentages than Douglas overall in our experiments. However, it seems safer to state that there are no predictable differences in germination of *Pteridium* in liquid and agar-solidified medium. This suggests that the higher yields of *P. aquilinum* and *A. phyllitidis* tissue in liquid than in agar-solidified medium observed by Douglas & Sheffield (1992) may have been due to higher growth rates of individuals in medium in liquid form. Our finding that fewer of the individuals growing in liquid medium go on to develop into 2-dimensional plates than on agar medium mirrors the data of Douglas (1994) relating to cell number. The latter study reported significantly fewer cells per gametophyte in 28 day-old individuals of the same species in liquid, than on agar-or agarose-solidified medium. This indicates that although early growth rate is enhanced, subsequent differentiation into the form usually adopted by *Pteridium* is perturbed by liquid medium. This is the reverse of the situation reported for *Platycerium bifurcatum* (Camloh, 1993). This may mean that the response to liquid is species- or ecotype-specific (as *P. bifurcatum* is an epiphytic species, and spores would conceivably land in more aqueous situations on tree bark than in the terrestrial sites colonised by *Pteridium*). Yields of *P. aquilinum* and *A. phyllitidis* are enhanced by using immobilised, rather than shake culture (Douglas & Sheffield, 1992) and perturbation of the development of these two species in liquid can be circumvented by the use of immobilised or air-lift cultures (Sheffield et al., 1997).

Differences in percentages of germinating spores from one population, experiment or culture to another are common in fern experiments (e.g. Pangua et al., 1999), and large differences in overall germination percentage is the rule in simultaneous experiments on different taxa in one set of conditions (e.g. Ranal, 1999). No attempt was made to identify the source of the variation between the species tested herein, because no effort had been made to refine the basic mineral medium or find preferred growth conditions for each fern. It is well known that surface sterilisation reduces percentage germination, and that spore age, storage conditions and culture conditions all have a great influence on fern spore viability (e.g. Miller, 1968; Raghavan, 1989; Camloh, 1993 & 1999; Sheffield, 1996). It is therefore unsurprising that the greatest differences in germination observed were attributable to the taxon tested. However, it is clear that for all four species tested here, whatever the background level of spore viability after collection, storage and surface sterilisation, the addition of sucrose enhances germination in liquid medium (see Fig. 1). This was not reported for *P. bifurcatum* by Camloh (1993), who found no promotive effect of sucrose on germination. Again, therefore, this may reflect species-

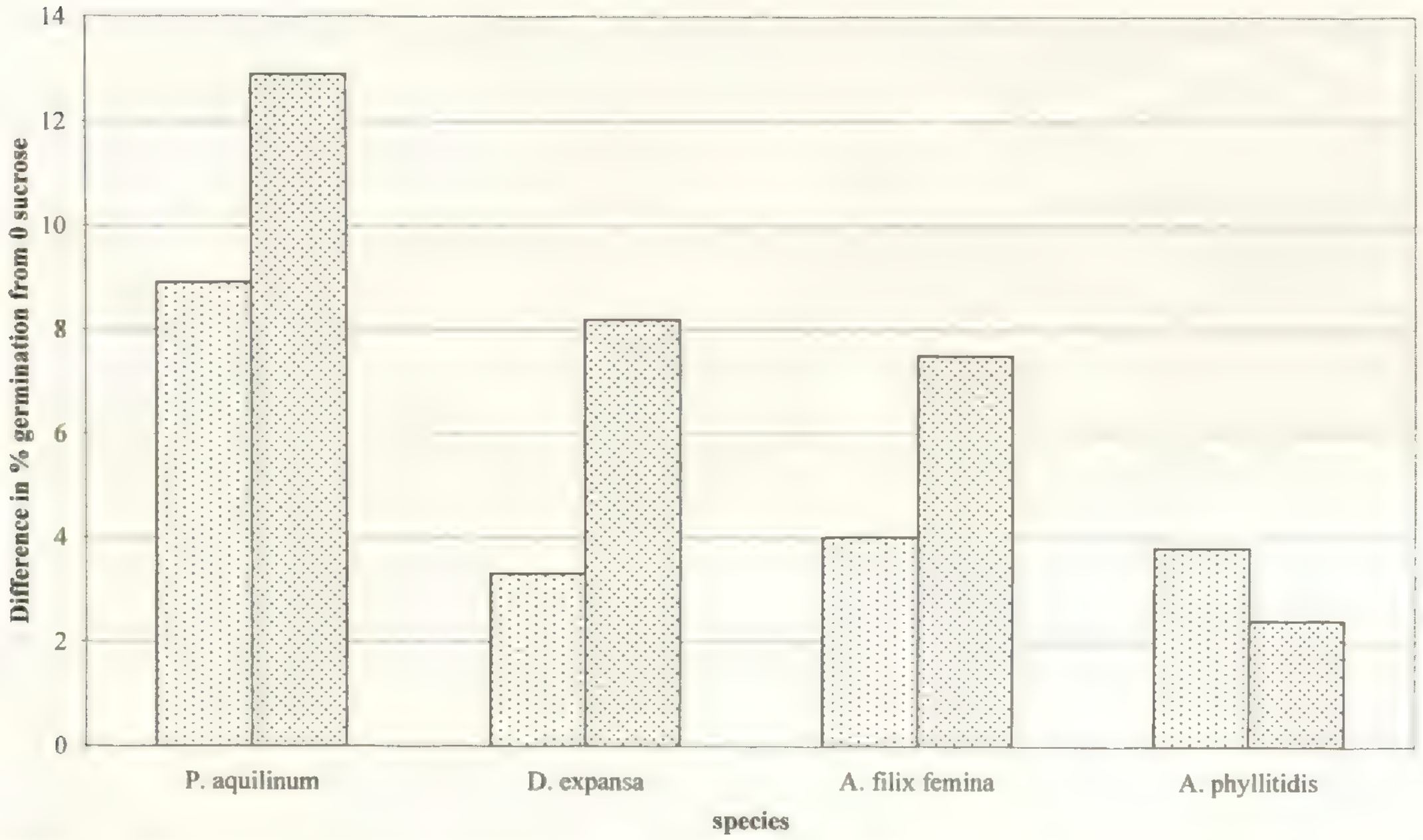

FIG. 1. Histogram of spore germination plotted as % differences between sucrose treatments (0.05M—dotted; 2M—diamond shading) and 0M sucrose controls. Order of species reflects increasing age of spores, youngest far left, oldest far right.

specific processes, or it may again be significant that *Platycerium* is epiphytic, and therefore very different from the terrestrial ferns tested in this study. The spores of *Platycerium* are also much larger than those of the species tested in the present study, so this difference may reflect different levels of storage carbohydrate. All the spores of *Platycerium* tested by Camloh may have had sufficient storage carbohydrate to germinate, in which case no increase in germination with added sucrose would be expected. It may also be that a different sugar or medium component would promote germination in this species. The spores Camloh used were stored before use, and it is known that levels of proteins as well as soluble sugars decline with storage of fern spores (Beri & Bir, 1993). It could be speculated, therefore, that the spores of *Platycerium* that fail to germinate in basal medium including sugar, might germinate if provided with amino acids.

Our finding that sucrose did not promote germination of *Anemia* spores as strongly as the other species may also reflect storage-related decline (see Fig. 1). The *Anemia* spores used here had been stored for many years longer than the other species. A pilot experiment carried out with the same spore sample five years earlier showed far greater promotion with sucrose (Douglas, 1994). In that experiment the germination percentages (s.e. in parentheses, n = 4) were as follows: 0M sucrose 53.1(6.7); 0.05M sucrose 72.5 (6.3); 0.2M sucrose 74.8 (3.4). The percentage germination without sucrose was very similar to that seen here (54.0), but the promotion of germination by sucrose was far more pronounced. This may suggest that during storage another cellular component had declined to limiting levels by the time the spores were retested.

The interaction between promotive effects of sucrose and length of time in storage should clearly be tested for individual spore samples, growth conditions and species. However, the results indicate that sucrose promotes germination in many ferns and would therefore be worth adding to media used to initiate cultures of a previously untested species.

This study examined the effects of sucrose on germination only. Effects of sucrose on subsequent growth of the species studied were not tested and appear to differ from one species to another. Camloh (1993) reported a stimulation of growth (in terms of cell number) using sucrose in medium used to culture *Platycerium*, while Douglas (1994) found evidence of growth inhibition (in terms of numbers achieving 2D growth) in *Anemia* and *Pteridium*. Fernández et al. (1997) reported that sucrose inhibited growth (fresh weight and gemma formation) in gametophytes of *Osmunda regalis*. Further experimentation with a wide range of species may reveal some reliable trends, but the current weight of evidence is on the side of deleterious effects of sucrose on growth of terrestrial species. This, coupled with the enhancement of growth of microbial contaminants which attends the use of media containing sugars, prompts us to suggest a 2-stage cultivation process in order to optimise yield/growth of cultured fern gametophytes. Germination should be effected in media containing sucrose, subsequent growth should employ immobilised or air-lift culture in media with no sucrose.

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Vernal Photosynthesis and Nutrient Retranslocation in *Dryopteris intermedia*

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ABSTRACT.—The value of preserving wintergreen fronds into the spring by forest understory fern species is unknown. In this study, net photosynthetic rates and nitrogen and phosphorus contents were monitored in a population of *Dryopteris intermedia* throughout a spring season to explore potential photosynthetic and retranslocational benefits of wintergreen fronds. Net photosynthesis occurred throughout the study indicating a potential for movement of fixed carbon from wintergreen fronds to other parts of the plant. Nitrogen and phosphorus content in the old fronds did not change through spring, thus no evidence for net retranslocation of these nutrients from wintergreen fronds to the rest of the plant was obtained. Maintenance of the wintergreen fronds may simply increase retention time and thus nutrient use efficiency of limiting nutrients. Other possible benefits of wintergreen fronds exist and should be investigated.

INTRODUCTION

The herbaceous understory layer of northern hardwood forests exhibits a plethora of leaf phenologies (Mahall and Bormann, 1978). One group of ecological importance is the wintergreen ferns. Species of this group maintain their fronds through winter and into spring only senescing after the current year's fronds begin unfolding. This contrasts with evergreen ferns, which maintain a set of fronds for more than one year. The value of over-winter maintenance of fronds has been hypothesized for some time (Monk, 1966; Chabot and Hicks, 1982; Moore, 1984; Van Buskirk and Edwards, 1995), yet tests of these hypotheses are limited (but see Minoletti and Boerner, 1993).

If the old fronds of *Dryopteris intermedia* (Muhl.) A. Gray are removed, the new fronds have a slower growth rate and begin to unfold later than if the old fronds are left intact (Van Buskirk and Edwards, 1995). Van Buskirk and Edwards (1995) hypothesized, in accordance with Moore (1984), that the old fronds either provided a photosynthetic or nutrient storage source to the plant that promoted the growth of the new fronds. Chabot and Hicks (1982), in reviewing the topic, also supported the view that wintergreen fronds provide a source of assimilation. The actual importance of the old fronds in this species has not been quantified.

The wintergreen fern *Polystichum acrostichoides* (Michx.) Schott is capable of year-round photosynthesis and nutrients are retranslocated from senescing old fronds back to the plant (Minoletti and Boerner, 1993). Also, the evergreen fern *Polypodium virginianum* L. was found to have photosynthetic peaks in the spring as a result of cool temperatures and high humidity, permitting more efficient CO_2 uptake (Gildner and Larson, 1992). Increased availability of soil nitrogen in spring (Murdoch and Stoddard, 1992, 1993; Zhang and Mitchell,

1995; Creed *et al.,* 1996; Mitchell *et al.,* 1996) may also help to increase photosynthetic rates (Chapin, 1980; Field and Mooney, 1986; Evans, 1989). These phenomena may be important for *Dryopteris intermedia,* making it energetically beneficial for this species to maintain photosynthetic tissues through spring. Likewise, retranslocation of nutrients from senescing organs, as shown in other herbs (DeMars and Boerner, 1997), can be of adaptive advantage by increasing the nutrient use efficiency of limiting nutrients (Chapin, 1980). Also, merely retaining old fronds for a longer period of time may increase nutrient use efficiency (Escudero et al., 1992). Thus, old fronds of *Dryopteris intermedia* may increase nutrient use efficiency through retranslocation of limiting nutrients or by extending the time over which a captured nutrient resource is used.

This study was designed to assess the photosynthetic and retranslocative benefits of wintergreen fronds in *Dryopteris intermedia.* The first objective was to measure net photosynthetic rates in old fronds through spring to determine if they are capable of photosynthesizing and therefore have the potential to provide a vernal fixed carbon source to the plant. The second objective was to measure nitrogen and phosphorus pool sizes through spring within component parts of *Dryopteris intermedia* and note changes in nutrient allocation that would indicate potential retranslocation.

Methods

A second growth northern hardwood forest was selected in the Catskill Mountains, NewYork, in which *Dryopteris intermedia* was abundant. Within each of twenty randomly located one-square-meter plots, a single wintergreen frond was randomly selected for study. Net photosynthetic measurements were made using an LCA-3 Carbon Dioxide Leaf Chamber Analysis System (Analytical Development Co., Ltd., 1988) between 10 AM and 2 PM on clear days. Air flow during measurements was 185 mL / minute. Ambient light levels, temperatures, and relative humidities were used in order to get an accurate reflection of the field net photosynthetic rates during spring. Measurement commenced as soon as the ground was free of snow (April 3, 1999) and was repeated through frond senescence (May 29, 1999). A total of three estimates of net photosynthetic rate in each of the twenty fronds were made through the spring. Also, cover and density of *Dryopteris intermedia* fronds were measured at bi-weekly intervals in these permanent plots from April 3 until May 29, 1999.

A plot of mean cover and density versus sample size indicated that at least 15 sample plots were necessary to accurately reflect the overall cover and density of *Dryopteris intermedia* within the stand. It would, however, have been logistically unfeasible and ecologically damaging to harvest so many plots at each study interval. Therefore, six additional randomly located sample plots were studied and harvested at each biweekly interval. In these plots, *Dryopteris intermedia* cover and frond density were measured and then all biomass was carefully harvested, placed in plastic bags, and stored in coolers

for transport to the lab. Roots and rhizomes were harvested by hand after scoring the perimeter of the plot with a shovel and carefully unearthing the root mat to collect the entirety of the fern belowground structures. Latex gloves were worn during harvest and subsequent handling in the lab to prevent contamination.

In the lab, the plant tissue samples were cleaned of soil using distilled, deionized water and placed in paper bags then dried at 60°C. Samples were separated into old fronds, belowground tissue, and (in the last two harvests) new fronds. The new fronds were not separated from the belowground tissue during the study until they had begun to unfurl. Next the samples were weighed and ground using a Wiley Mill to pass through a 1mm screen. These samples were then analyzed for N by Kjeldahl digestion (Bickelhaupt and White 1982). Lastly they were subjected to microwave digestion with nitric acid using a CEM MDS 81D (Gilman, 1988) and analyzed for P using a Perkin-Elmer Optima 3300 DV ICP.

Fern component specific regressions for biomass on a meter square of land area basis were determined for the 6 harvest plots based on cover and density of fronds at each sample date. Using this regression it was possible to estimate biomass in the 20 permanent plots thereby providing a more accurate estimate of stand level biomass than would be given by the six harvest plots, alone. Multiplying N and P concentration (from the analytical procedures) by biomass per fern component (from the regressions) permitted an estimate of N and P content on a meter square basis. Standard errors of these combined estimates were determined using equations from Avery and Burkhart (1994).

All statistical analyses were performed using SAS version 6.12 (SAS Institute, Inc., 1997). Differences in net photosynthetic rate among the three estimates were found using ANOVA followed by a Tukey's Honestly Significant Difference Test ($\alpha = 0.05$). Differences in N concentration and content, P concentration and content, and biomass among the fern components and harvest times were isolated by ANOVA followed by a Tukey's Honestly Significant Difference Test ($\alpha = 0.05$).

Results

Wintergreen fronds were capable of positive net photosynthesis throughout the spring season (Figure 1). The highest rate of net photosynthesis was observed early in spring immediately after snowmelt. Remarkably, positive net photosynthesis was evident even in later stages of senescence (browned and withering) toward the end of the study.

Nitrogen content in the wintergreen and new fronds exhibited no change during the spring season (Figure 2a). Nitrogen values for belowground structures, on the other hand, first increased and subsequently decreased (Figure 2a). These belowground structures contained the majority of the N in the plants, particularly early on.

The nitrogen concentration of the different plant components did not change significantly through spring (Figure 2b). As the new fronds were not yet fully

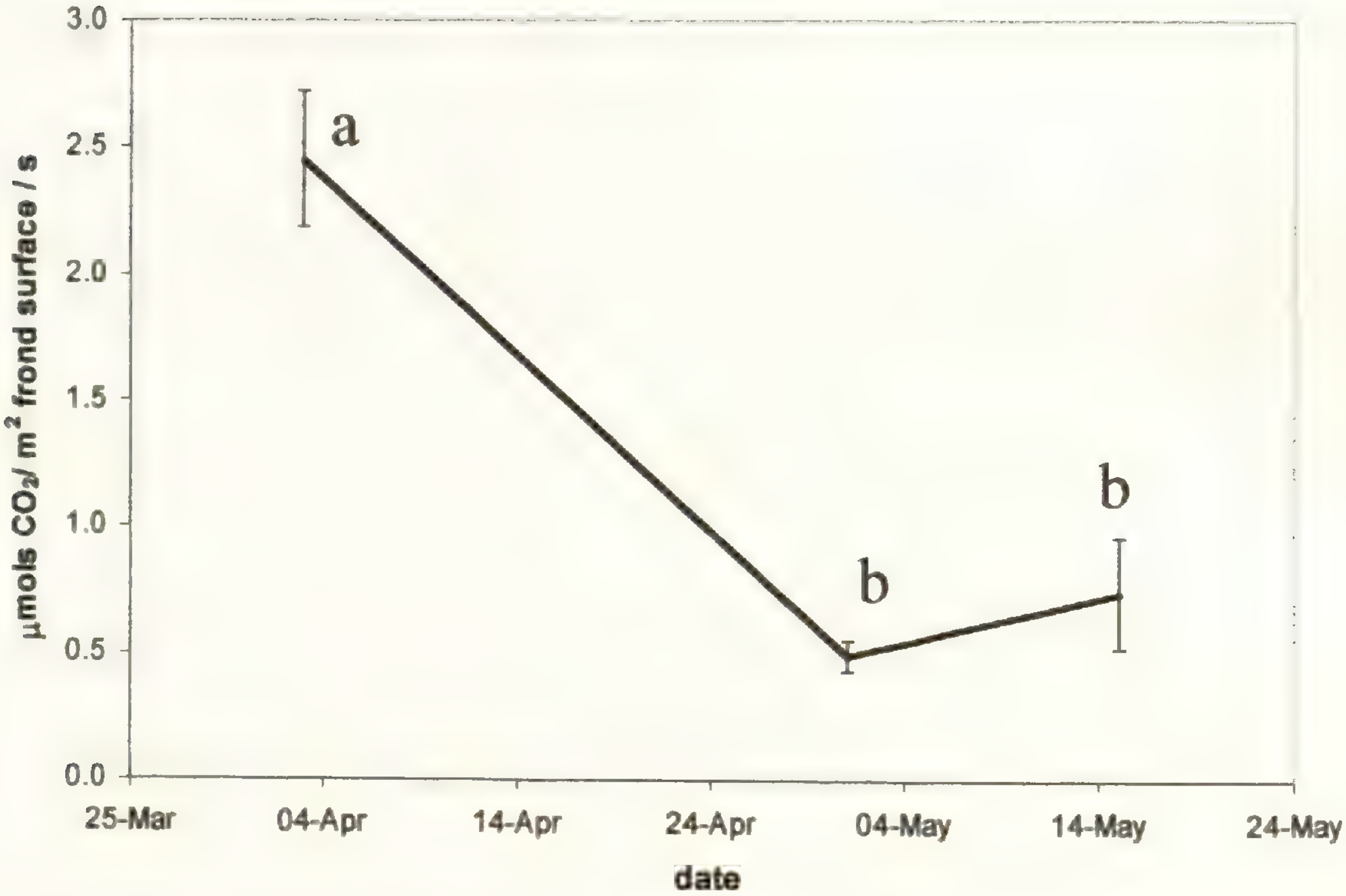

FIG. 1. Net photosynthesis through spring. Means with the same letter were not significantly different at $\alpha = 0.05$. Error bars indicate one standard error above and below the mean.

expanded at the end of the study, their N concentration was markedly higher than that of the other fern components. Figure 2c shows biomass of the fern components through time. Although the variations in biomass through time were not statistically significant, they do follow the pattern seen for N and P content (see below).

The trend in phosphorus content (Figure 3a) was similar to that for nitrogen. Wintergreen and new fronds remained constant in their P content while belowground structures increased and then decreased with time. The phosphorus concentration trend (Figure 3b) was very similar to that of N concentration.

DISCUSSION

PHOTOSYNTHETIC RATE.–It is clear that the wintergreen fronds are photosynthesizing during spring. Rates observed in this study are comparable to autumn, winter, and spring rates in *Polystichum acrostichoides* (Minoletti and Boerner, 1993) and slightly higher than rates under experimental conditions in other shade-tolerant fern species (Ludlow and Wolf, 1975). Data for summer photosynthetic rates are unavailable for comparison. As the highest rates of

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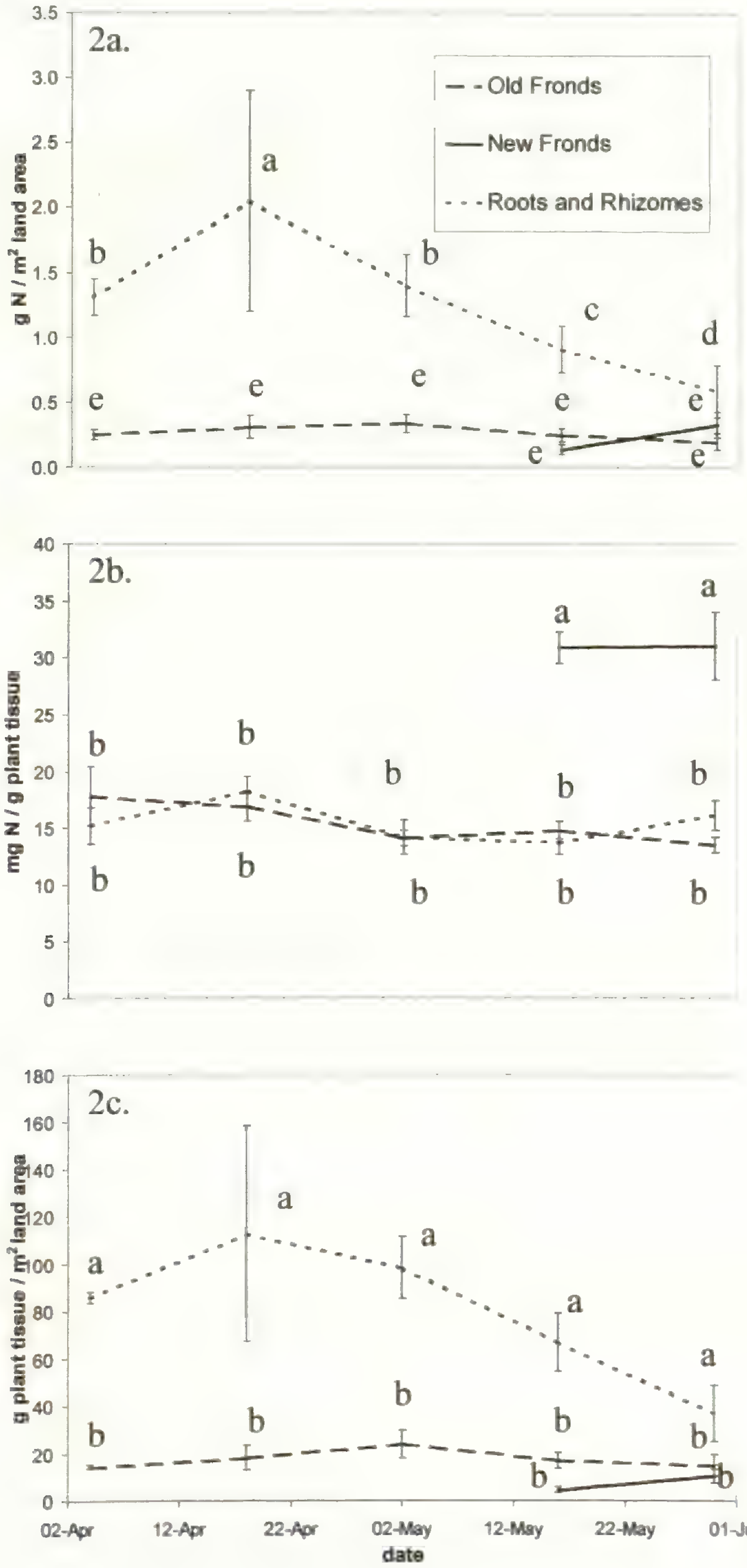
FIG. 2a. Nitrogen content of fern components through spring. 2b. Nitrogen concentration of fern components through spring. 2c. Biomass of fern components through spring. Means with the same letter were not significantly different at $\alpha = 0.05$. Errors bars indicate one standard error above and below the mean.


2a.
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
g N / m² land area
Old Fronds
New Fronds
Roots and Rhizomes
a
b
b
c
d
e
e
e
e
e
e
2b.
40
35
30
25
20
15
10
5
0
mg N / g plant tissue
a
a
b
b
b
b
b
b
b
b
b
2c.
180
160
140
120
100
80
60
40
20
0
g plant tissue / m² land area
a
a
a
a
a
b
b
b
b
b
b
02-Apr
12-Apr
22-Apr
02-May
12-May
22-May
01-Jun
date

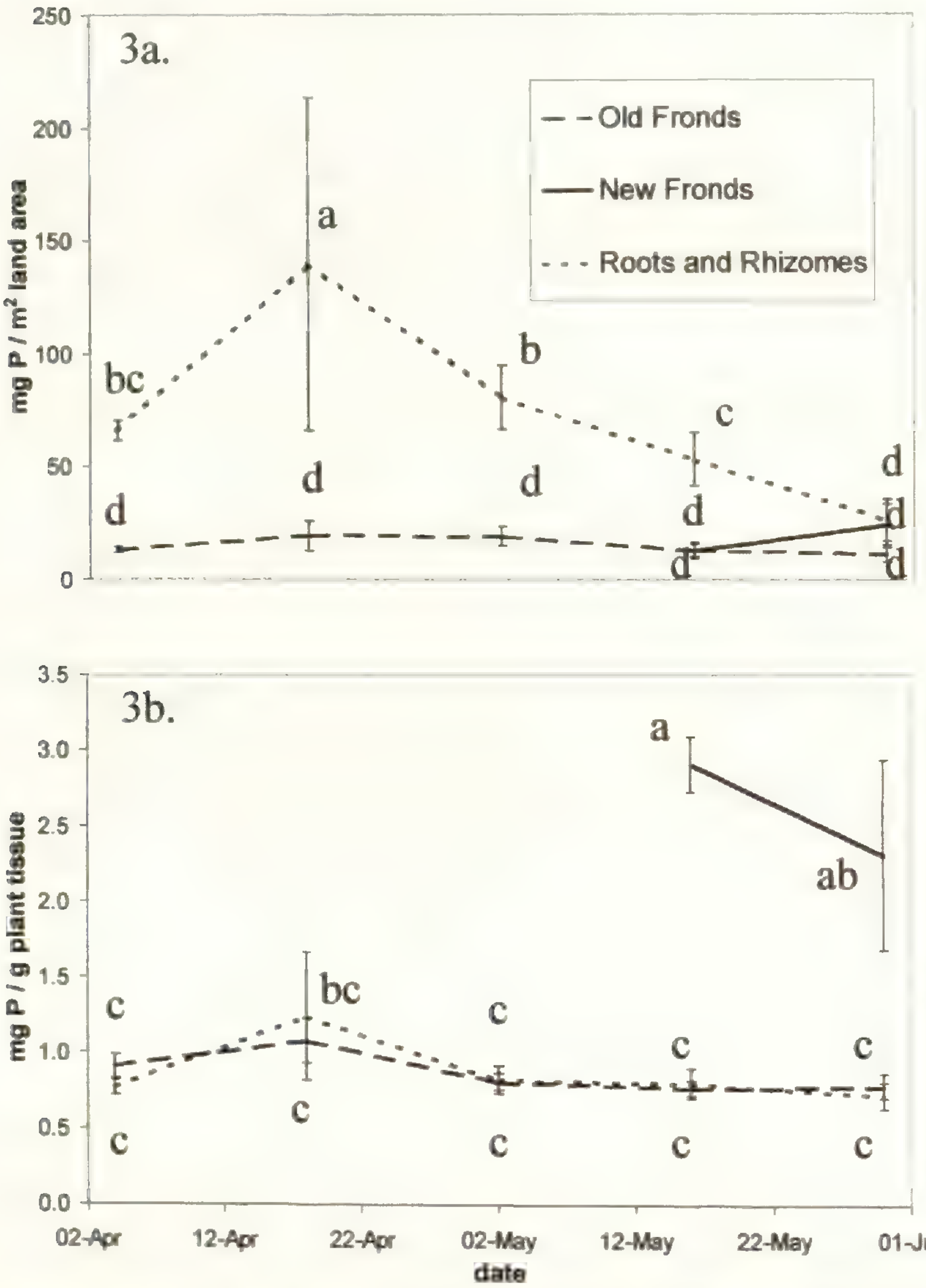


FIG. 3a. Phosphorus content of fern components through spring. 3b. Phosphorus concentration of fern components through spring. Means with the same letter were not significantly different at $\alpha = 0.05$. Error bars indicate one standard error above and below the mean.

net photosynthesis are observed early in the season (Figure 1), it is possible that photosynthesis may be occurring in these fronds during periods of the winter as well (Salisbury 1984, 1985), so long as light sufficiently penetrates the snow pack and temperatures permit metabolic activity.

The capacity to photosynthesize during spring may be important to annual net energy capture in *Dryopteris intermedia*. It may be critical to an understory species that usually receives low light levels in the shade of canopy species during the summer season (Anderson, 1964; Federer and Tanner, 1966; Hutchison and Matt, 1977) to take advantage of high light conditions prior to canopy leaf out. Spring ephemerals, species that develop above ground early in spring and senesce with canopy leaf out, are capable of high rates of photosynthesis during spring as compared to summer rates (Harvey 1980) and are adapted to

growth and reproduction during this period of high light prior to canopy leaf out. Also, some disturbance-adapted species can acclimate to periods of high light (Brach et al., 1993) and increase their photosynthetic rate. Since *Dryopteris intermedia* does not respond to these periods of high light (Brach et al., 1993), maintaining year-round photosynthetic capacity may be critical to optimizing productivity. A comparison of spring and summer photosynthetic rates would be valuable to test this hypothesis.

It is not clear, however, that the C fixed by the wintergreen fronds is translocated to the new fronds or to any other part of the fern. Carbon gained during the spring period may be lost to soil microbes as the fronds decompose and not be of any immediate benefit to the plant. The non-significant pattern of increased belowground biomass in early spring (Figure 2c) suggests that carbon may be moving from the photosynthesizing fronds into the belowground tissue. A critical next step would be to conduct a tracer study to isolate the intermediate and eventual sinks for the spring-fixed carbon.

NITROGEN AND PHOSPHORUS POOLS.—As is typical for many herbaceous perennial plants (Zavitkovski, 1976), much more biomass is present belowground in *Dryopteris intermedia* than in aboveground components (Figure 2c). The mean root:shoot for biomass is roughly 4:1 through the spring. Unlike biomass, the concentration of N and P does not differ between mature parts of the plant. In fact the vernal constancy in N and P concentration and content in the wintergreen fronds suggests that no net mineral nutrient retranslocation to the rest of the plant is occurring with frond senescence. This contrasts with nutrient retranslocation patterns seen during senescence in other herbaceous understory plants (DeMars and Boerner, 1997). Short senescence times can result in greater retranslocation efficiency (del Arco et al., 1991). Slow senescence of the wintergreen fronds (from snowfall through mid spring) may preclude efficient retranslocation in this species. Therefore, maintaining wintergreen fronds as storage organs may not benefit the plant in the retranslocation and subsequent use of the nutrients.

PHOTOSYNTHETIC NUTRIENT USE EFFICIENCY.—Maintenance of the wintergreen fronds does, however, increase the nutrient retention time of N and P, thereby increasing nutrient use efficiency (Escudero et al., 1992) of these often limiting nutrients (Chapin, 1980; Vitousek and Howarth, 1991; Yanai, 1992). How much of an increase in nutrient use efficiency does the maintenance of wintergreen fronds in *Dryopteris intermedia* provide? Often nutrient use efficiency is defined as the inverse of the nutrient concentration (Vitousek, 1982; Shaver and Mellilo, 1984; Hirose and Werger, 1994; Minotta and Pinzauti, 1996; Fisk et al. 1998). This definition does not take into account energy expended for reproductive and maintenance efforts, only those expended for growth. Therefore, simply examining the inverse of the nutrient concentration may ignore significant uses of nutrients. A more useful assessment of nutrient use efficiency examines total carbon fixed per unit nutrient termed the Potential Photosynthetic Nutrient Use Efficiency (Field and Mooney, 1986; Hirose and Werger, 1994).

In this study, ambient light intensities were used to assess net photosynthetic rates since these rates are a better representation of the actual benefit of maintaining wintergreen fronds than would be light saturated net photosynthetic rates. Photosynthetic nutrient use efficiencies (PNUE) were 9.80, 1.44, and 2.96 μmols CO_2 / g / second for nitrogen on April 3, April 31, and May 14, respectively. For phosphorus PNUE were 0.19, 0.03, and 0.06 μmols CO_2 / mg / second on April 3, April 31, and May 14, respectively. Vitousek (1982) points out that one way to improve nutrient use efficiency is by fixing C for a longer period of time. In this case, springtime photosynthesis in wintergreen fronds clearly improves nutrient use efficiency since without this springtime photosynthesis PNUE would be zero until the new fronds began photosynthesizing.

In summary, vernal net photosynthesis does occur in the wintergreen fronds of *Dryopteris intermedia*, however it is not clear that the fixed carbon is translocated to the rest of the plant, and a tracer study would be useful to test this possibility. No evidence of N and P retranslocation from senescing wintergreen fronds to the rest of the fern plant was observed. Maintenance of wintergreen fronds may simply increase photosynthetic nutrient use efficiency of nitrogen and phosphorus. Future studies should examine the long-term fate of carbon and mineral nutrients held within senescing fronds and compare spring and summer photosynthetic rates in this species.

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Phylogenetic Placements of *Loxoscaphe thecifera* (Aspleniaceae) and *Actiniopteris radiata* (Pteridaceae) Based on Analysis of *rbcL* Nucleotide Sequences

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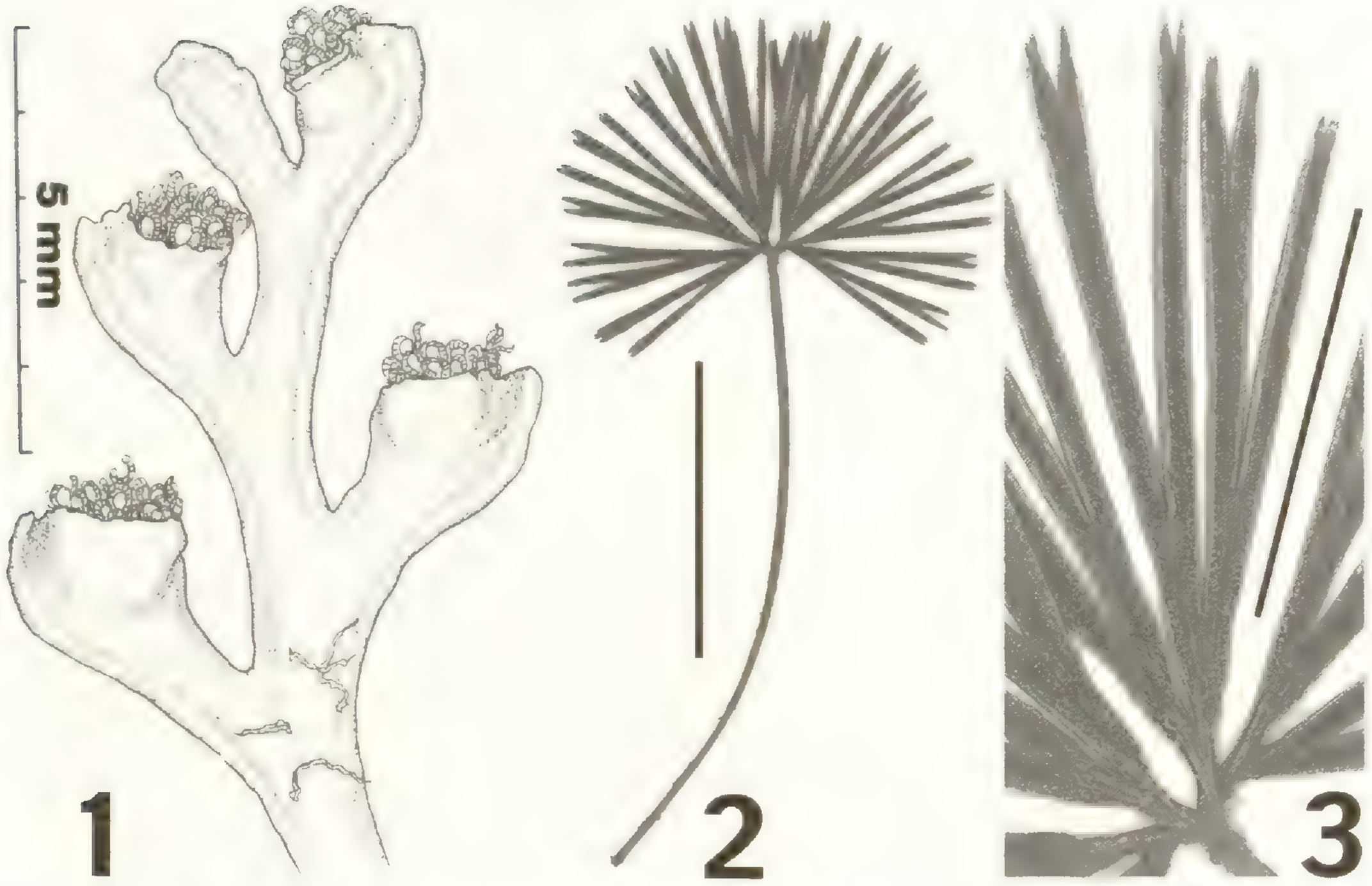
ABSTRACT.—Nucleotide sequences of the chloroplast-encoded *rbcL* gene were determined for *Loxoscaphe thecifera* and *Actiniopteris radiata* and used in maximum parsimony cladistic analyses to determine their phylogenetic positions in the context of a broad range of advanced fern taxa. *Loxoscaphe* nested firmly within the Aspleniaceae, and *Actiniopteris* was placed with *Onychium* in the Pteridaceae. To help resolve conflicting contemporary treatments that either subsume *Loxoscaphe* species within *Asplenium* or segregate them as an independent genus, the *rbcL* sequence of *L. thecifera* was subjected to a more focused analysis involving all *rbcL* sequences available to represent the taxonomic diversity of Aspleniaceae. *Loxoscaphe thecifera* was sister to *Asplenium griffithianum* + *prolongatum*, robustly and surprisingly nested within the clade of *Asplenium* species recognized as *Asplenium* section *Thamnopteris*, a group accepted by some as the segregate genus *Neottopteris*. These results indicate that there is presently no phylogenetic justification for accepting *Loxoscaphe* as a genus independent of *Asplenium*. Similarly *Actiniopteris radiata*, recently moved from the cheilanthoid to the taenitidoid group of Pteridaceae, was subjected to a more focused analysis in the context of an expanded set of cheilanthoid and taenitidoid species that included the first use of an *rbcL* sequence from the genus *Anogramma* and newly sequenced species of *Onychium* and *Pteris*. *Actiniopteris* is robustly grouped with two *Onychium* species in a clade sister to traditional taenitidoids and deeply separated from the cheilanthoids, supporting affinities previously suggested by spore morphology.

Loxoscaphe T. Moore is a genus of epiphytic species segregated from *Asplenium* L. by many contemporary authors (e.g., Crabbe et al., 1975; Pichi Sermolli, 1977; Smith, 1981; Mickel and Beitel, 1988; Lellinger, 1989), although others (Tryon and Tryon, 1982; Kramer and Viane, 1990; Tryon and Stolze, 1993) reject this segregation, instead subsuming *Loxoscaphe* species within the genus *Asplenium*. Mickel and Beitel (1988) expressed the segregationist view in stating that "*Loxoscaphe* is a genus of four or five species, one in the neotropics, two in Africa (one sometimes considered only varietally distinct from the neotropical species), and two in the South Pacific and Indonesia. Although it is most closely allied to and sometimes placed in synonymy under *Asplenium*, *Loxoscaphe* superficially more closely resembles *Odontosoria* in the pocketlike sori terminal on the narrow blade segments, but the small fronds, heavy texture, and epiphytic habit readily distinguish it from that genus." Its sori in pouches near the margin of the leaf segments (Fig. 1) give it the appearance of a *Davallia*, in which genus most of the older species of *Loxoscaphe* were first described, as noted by Copeland (1947). In commenting on the generic placement of *Loxoscaphe thecifera* (Kunth) T. Moore (as *Asplenium*) in their treatment of the ferns of Peru, Tryon and Stolze (1993)



FIGS. 1–3. Illustrations of *Loxoscaphe thecifera* and *Actiniopteris radiata*. 1. Apex of a pinna of *Loxoscaphe thecifera* drawn from *Holm-Nielson et al. 4110*, Ecuador (F) to show the pouch-like indusia characteristic of *Loxoscaphe*. Reproduced from Tryon and Stolze (1993) with permission from the Field Museum of Natural History. 2. Adaxial view of a sporophyll from a cultivated plant of *Actiniopteris radiata* (*Gastony 12–97–102* IND from Tanzania), showing the distinctive, flabellate lamina of this xeromorphic fern. Scale bar = 5 cm. 3. Magnified view of fascicle of segments from the right half of the lamina in Fig. 2, showing continuous, membranous, marginal indusia partly covering the submarginal sori. Scale bar = 2 cm.

noted that the group of *Asplenium* species often placed in the genus *Loxoscaphe* are quite distinctive in their indusia, which are generally pouch- or cup-shaped and occur near the segment margin. They noted that by means of this indusial character state, *Asplenium theciferum* (Kunth) Mett. cannot be confused with any other *Asplenium* in Peru, their area of coverage. Their retention of the *Loxoscaphe* group within *Asplenium* was influenced by their observation that the sori of a few Old World species in the group intergrade to a more typical *Asplenium* sorus.

The global analysis of fern phylogeny by Hasebe et al. (1995) based on *rbcL* nucleotide sequences did not include a representative of the *Loxoscaphe* species group, but the results of that study do provide a context in which the generic relationships of *Loxoscaphe* can now be assessed. Doing that is one goal of this paper. To focus the reader's attention on *Loxoscaphe* and other relevant groups sometimes proposed for generic segregation from *Asplenium*, the following text and figures 5 and 6 refer to these taxa by their names in segregate genera. This is simply an attention-focusing device and does not signal our taxonomic conclusions, which are sometimes contrary to the seg-

regate nomenclature. Alternative nomenclatures for these groups are noted in the lists of species in MATERIALS AND METHODS and in Table 1.

Actiniopteris Link was regarded as an Old World genus of cheilanthoid affinities by Tryon and Tryon (1982) who placed it (as *Actinopteris*) in Pteridaceae tribe Cheilantheae. Later, Tryon et al. (1990) shifted *Actiniopteris* from cheilanthoid to taenitidoid affinity by placing it in Pteridaceae subfamily Taenitidoideae, without explanation. *Actiniopteris radiata* (Sw.) Link, with an unusual fan-shaped lamina (Fig. 2) bearing linear, submarginal sori with continuous, membranous, marginal indusia (Fig. 3), is widespread within the Afro-Indian distribution of its genus (Tryon et al., 1990). It occurs, for example, throughout tropical Africa where hot and dry conditions prevail (Jacobsen, 1983). Continuing the ongoing efforts of the first author to decipher phylogenetic relationships among the lineages of cheilanthoid ferns (Gastony and Rollo, 1995, 1998), a second goal of the present study is inferring the subfamilial affinities of *Actiniopteris* on the basis of *rbcL* data.

The first author encountered epiphytic plants of *Loxoscaphe thecifera* on the rim of the Ngorongoro Crater and terrestrial plants of *Actiniopteris radiata* in fissures of the limestone cap bordering Olduvai Gorge, both during a recent trip to Tanzania. Materials suitable for DNA-level analyses of these species were obtained. The resulting *rbcL*-based phylogenetic analyses determine the phylogenetic relationships of these species and provide a fresh perspective for addressing the generic disposition of *Loxoscaphe*.

MATERIALS AND METHODS

Total genomic DNA of *Actiniopteris radiata* and *Loxoscaphe thecifera* was extracted by the DNeasy method (Qiagen, Inc., Valencia, CA) from silica-gel dried leaves of sporophytes collected in nature (Table 1), purified with the Elu-Quick DNA purification kit (Schleicher & Schuell, Keene, NH), and quantified to 20 ng/μl with a Hoeffer Scientific fluorometer. DNA of the other species in Table 1 was obtained as follows: *Anogramma lorentzii* from gametophytes grown from spores of the Brazilian sporophyte voucher, *Pteris cretica* from fresh, field-collected sporophytes, and *Onychium lucidum* from sporophytes grown from spores of the Chinese voucher. Polymerase Chain Reaction (PCR) amplification of the *rbcL* gene was as reported in Gastony and Rollo (1995) except that for *Loxoscaphe thecifera* the hot start PCR method of Gastony and Ungerer (1997) was used with the following specifications: 0.8 μl @20 ng/μl of genomic template DNA was added to 8.62 μl distilled H_2O, 3.6 μl of Master Mix (see components below), 0.24 μl of 10 μM forward primer 1F, and 0.24 μl of 10 μM reverse primer 1351R (primers 1F and 1351R as in Gastony and Rollo, 1995). In PCR, these reagents were heated to 96°C for 5 min to denature the templates before adding 1.5 μl Taq polymerase ("hot start") during a 5 min period at 72°C. The final concentration of the hot start Master Mix was 2 mM $MgCl_2$, 30 mM tricine, 50 mM KCl, 100 μM of each dNTP, and 5% acetamide. In all cases PCR was carried out in an MJ Research Thermal Cycler programmed as follows (after the initial 96°C denaturation or

TABLE 1. Sources of material and GenBank accession numbers for *rbcL* sequences of taxa used in this study. Species for which data were published in the Appendix of Hasebe et al. (1995), Table 2 of Murakami et al. (1999a), or Table 1 of Murakami et al. (1999b) are not repeated here (see Materials and Methods for lists of those species). All collections below are vouchered at IND.

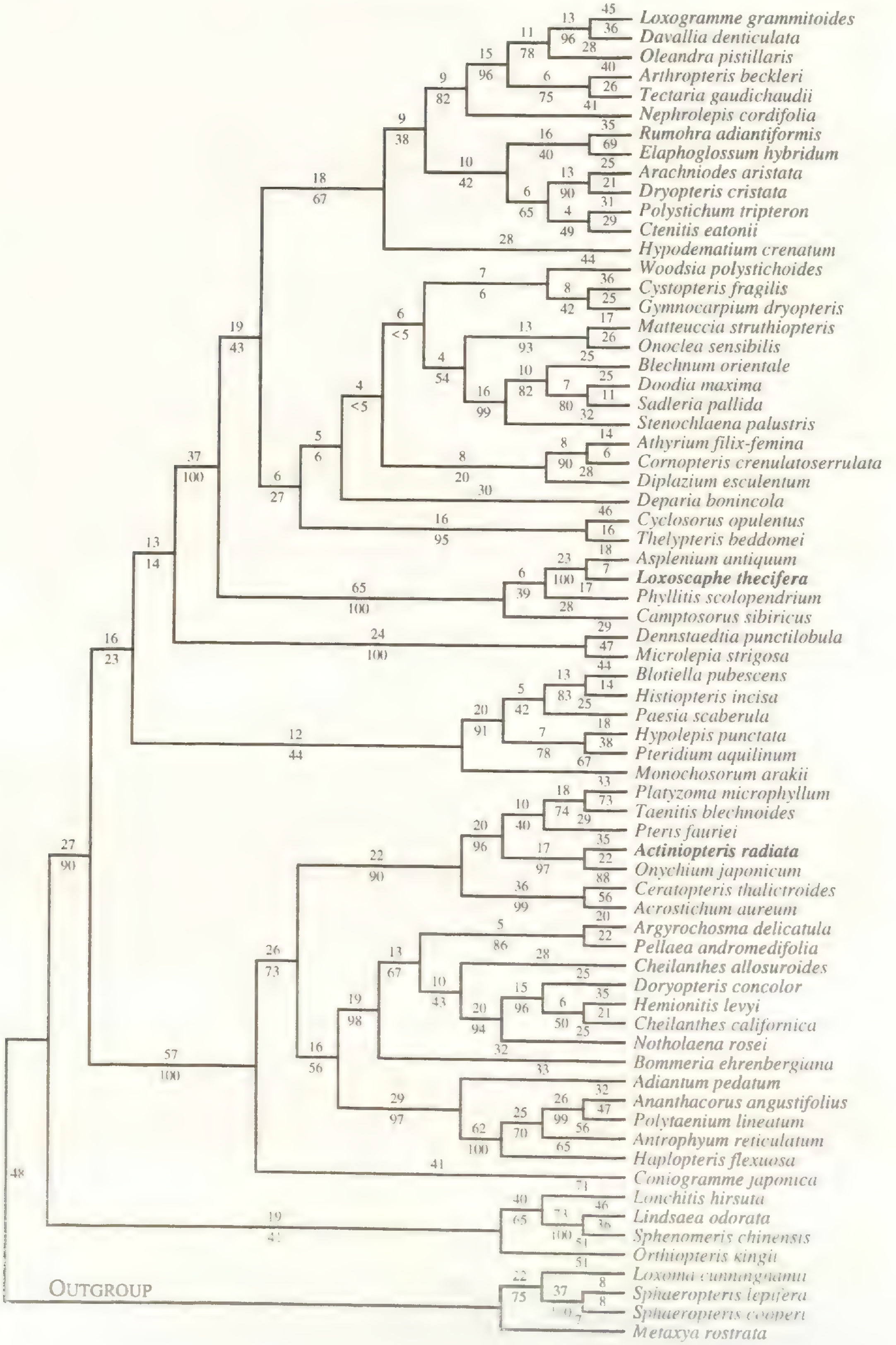
Species	Provenance	Collection (Voucher)	GenBank accession no.	Figures in this study
Actiniopteris radiata (Sw.) Link	Olduvai Gorge, Tanzania	*Gastony 12-97-102*	AF336100	4, 7
Anogramma lorentzii (Hieron.) Diels	Rio Grande do Sul, Brazil	*Sehnem s.n.*, 10/10/72	AF336102	7
Asplenium theciferum (Kunth) Mett. ex *Loxoscaphe thecifera* (Kunth) T. Moore	Ngorongoro Crater, Tanzania	*Gastony 12-97-101*	AF336099	4, 5, 6
Cheilanthes californica (Hook.) Mett.	California, U.S.A.	*Kirkpatrick s.n.*	AF336101	4, 7
Onychium lucidum Spreng.	Yunnan, China	*Li & Xiang S-8L*	AF360359	7
Pityrogramma calomelanos (L.) Link	Oaxaca, Mexico	*Yatskievych & Gastony 89-251*	AF336103	7
Pityrogramma trifoliata (L.) R. M. Tryon	Oaxaca, Mexico	*Yatskievych & Gastony 89-252*	AF336104	7
Pteris cretica L.	Hidalgo, Mexico	*Yatskievych & Gastony 89-208*	AF360360	7

after the hot start addition of Taq): 40 cycles of 94°C for 1 min, 45°C for 1 min, 72°C for 2 min, followed by a 6 min final extension at 72°C and storage at 4°C. PCR yielded a 1381 base pair fragment of the *rbcL* gene, including the forward and reverse primer regions. With the suite of forward and reverse sequencing primers listed in Table 2 of Gastony and Rollo (1995), we were able to read 1325 bp of sequence between the PCR primers in both directions. Sequencing was carried out with an ABI automated sequencer according to manufacturer's protocols, and the contigs were assembled and read with Sequencher™ 3.1.1 (Gene Codes Corporation, Ann Arbor, MI).

To determine the phylogenetic relationships of *Loxoscaphe thecifera* and *Actiniopteris radiata*, their 1325 bp *rbcL* sequences (Table 1) were analyzed cladistically with PAUP* 4.0b4a (Swofford, 1998) in three separate analyses as follows.

In the first analysis, the *Loxoscaphe* and *Actiniopteris* sequences were combined with those of a large subset of the species used in the global analysis of fern phylogeny of Hasebe et al. (1995) to determine where *Loxoscaphe* and *Actiniopteris* would probably have been positioned if their *rbcL* sequences had been included in that 1995 analysis. In reference to Fig. 3 of Hasebe et al. (1995), the *rbcL* sequences of all taxa from *Orthiopteris* through *Loxogramme* were obtained from GenBank (except for *Cornopteris crenulatoserrulata*, *Deparia bonincola*, and *Hypodematium crenatum*, which were provided by Mitsuyasu Hasebe) and used for the ingroup for this first analysis along with the new sequences of *Loxoscaphe* and *Actiniopteris* and that of *Cheilanthes calif-ornica* (Table 1), an American cheilanthoid sometimes segregated into the genus *Aspidotis* and shown by Gastony and Rollo (1998) to have Asian affinities. This broad spectrum of taxa includes dennstaedtioids, lindsaeoids, *Davallia*, taenitidoids, cheilanthoids, and other advanced fern taxa in order to permit free positioning of *Loxoscaphe* and *Actiniopteris* wherever their *rbcL* affinities might reasonably lie. As outgroup for this analysis the GenBank *rbcL* sequences of four taxa (*Loxoma*, *Cyathea* [represented by *Cyathea lepifera*, more appropriately named *Sphaeropteris lepifera* as in our Fig. 4], *Sphaeropteris*, and *Metaxya*) from the clade sister to this ingroup in Fig. 3 of Hasebe et al. (1995) were selected. Thus, 69 taxa (65 ingroup plus 4 outgroup, Fig. 4) were used in this first analysis with PAUP*4.0b4a (Swofford, 1998) on a Macintosh PowerPC G4 350MHz with the following specifications: maximum parsimony, heuristic search, all characters equally weighted, starting tree by stepwise addition, random addition sequence with 1000 replicates, random starting seed number generated by the program, TBR branch swapping, MulTrees, and steepest descent. Of the 1325 characters, 504 were parsimony-informative. The positions of *Loxoscaphe* and *Actiniopteris* determined by this first analysis permitted subsequent selection of appropriate new ingroups and outgroups for each of them in more detailed analyses of their respective relationships in the second and third analyses.

The first analysis placed *Loxoscaphe* with *Asplenium* and its relatives, and *Actiniopteris* in the clade of Pteridaceae plus Vittariaceae (Fig. 4). For a more focused analysis of the *rbcL* relationships of *Loxoscaphe* within Aspleniaceae


Loxogramme grammitoides
Davallia denticulata
Oleandra pistillaris
Arthropteris beckleri
Tectaria gaudichaudii
Nephrolepis cordifolia
Rumohra adiantiformis
Elaphoglossum hybridum
Arachniodes aristata
Dryopteris cristata
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Lonchitis hirsuta
Lindsaea odorata
Sphenomeris chinensis
Orthiopteris kingii
Loxoma cunninghamii
Sphaeropteris lepifera
Sphaeropteris cooperi
Metaxya rostrata
OUTGROUP

the clade from *Asplenium* through *Camptosorus* of Fig. 4 was therefore selected as a new ingroup, to which were added Aspleniaceae sequences available in GenBank (27 total ingroup taxa, Fig. 5), and a subset of seven taxa in the sister clade from *Loxogramme grammitoides* through *Thelypteris beddomei* (Fig. 4) was selected as the outgroup (Fig. 5). Most of these ingroup species were used in a recent *rbcL* study of the phylogeny of Aspleniaceae (Murakami et al., 1999b), the results of which are seen in Fig. 1 of that study. Use of generic names *Camptosorus*, *Neottopteris*, *Phyllitis*, and *Hymenasplenium* in discussing our results in Figs. 5 and 6 below facilitates comparison with Fig. 1 of Murakami et al. (1999b). *Boniniella ikenoi* of Murakami et al. (1999b) Fig. 1 is reported here under its name as a species of *Hymenasplenium*, *H. cardiophyllum*. Of the 1325 characters in this data set, 275 were parsimony-informative, probably reflecting the fact that the GenBank sequences of many of the *Asplenium* species used in the ingroup for Figs. 5 and 6 were much shorter than those generated in our lab, and their 5′ and 3′ end truncations relative to ours were treated as missing data.

Similarly, for a more focused analysis of the *rbcL* relationships of *Actiniopteris*, the clade from *Platyzoma microphyllum* through *Haplopteris flexuosa* of Fig. 4 was selected as a new ingroup. To these were added (Table 1) species of the putatively taenitidoid (Tryon and Tryon, 1982; Tryon et al., 1990) genera *Pityrogramma* (*P. calomelanos* and *P. trifoliata*, both previously used by Gastony and Rollo, 1998) and *Anogramma* (*A. lorentzii*, new here), and additional species of *Onychium*, *Pteris*, and *Cheilanthes* (*O. lucidum* and *P. cretica*, both new here; *C. lanosa* previously used by Gastony and Rollo, 1995, 1998), yielding 26 total ingroup taxa (Fig. 7, Table 1). As outgroup to this new *Actiniopteris* ingroup the sister clade *Coniogramme* of Fig. 4 was used, plus two basal taxa from the next most sister clade, viz. *Monachosorum arakii* and *Microlepia strigosa* (Fig. 4). Of the 1325 characters in the data set, 374 were parsimony-informative. Both of these more focused analyses were carried out in the same manner as the first analysis.

The bootstrap option in PAUP* was used to assess support for clades revealed in each of the three analyses. Bootstrapping for the first analysis was

←

FIG. 4. Single most parsimonious tree placing *Loxoscaphe thecifera* and *Actiniopteris radiata* (bold) within the context of taxa seen in Fig. 3 of Hasebe et al. (1995), with the addition of *Cheilanthes californica*. Based on the findings in Fig. 3 of Hasebe et al. (1995), to which the topology of this tree is comparable, the bottom clade from *Loxoma* through *Metaxya* served as outgroup (with the name *Cyathea lepifera* of Hasebe et al. [1995] corrected to the more accurate designation *Sphaeropteris lepifera*). Crane et al. (1995) are followed in changing the name *Vittaria flexuosa* of Hasebe et al. (1995) to *Haplopteris flexuosa*. This tree was generated by a heuristic search with 1000 random addition sequence replicates used to obtain the starting trees. Numbers of synapomorphies supporting each clade and autapomorphic branch lengths for terminal taxa are indicated above the lines. Bootstrap percentages based on 1000 replicates of five random addition sequence replicates each are provided below the lines. The placements of *Loxoscaphe thecifera* and *Actiniopteris radiata* determined here directed choices of more focused ingroups and outgroups for the analyses reported in Figs. 5–7, as explained in the text.

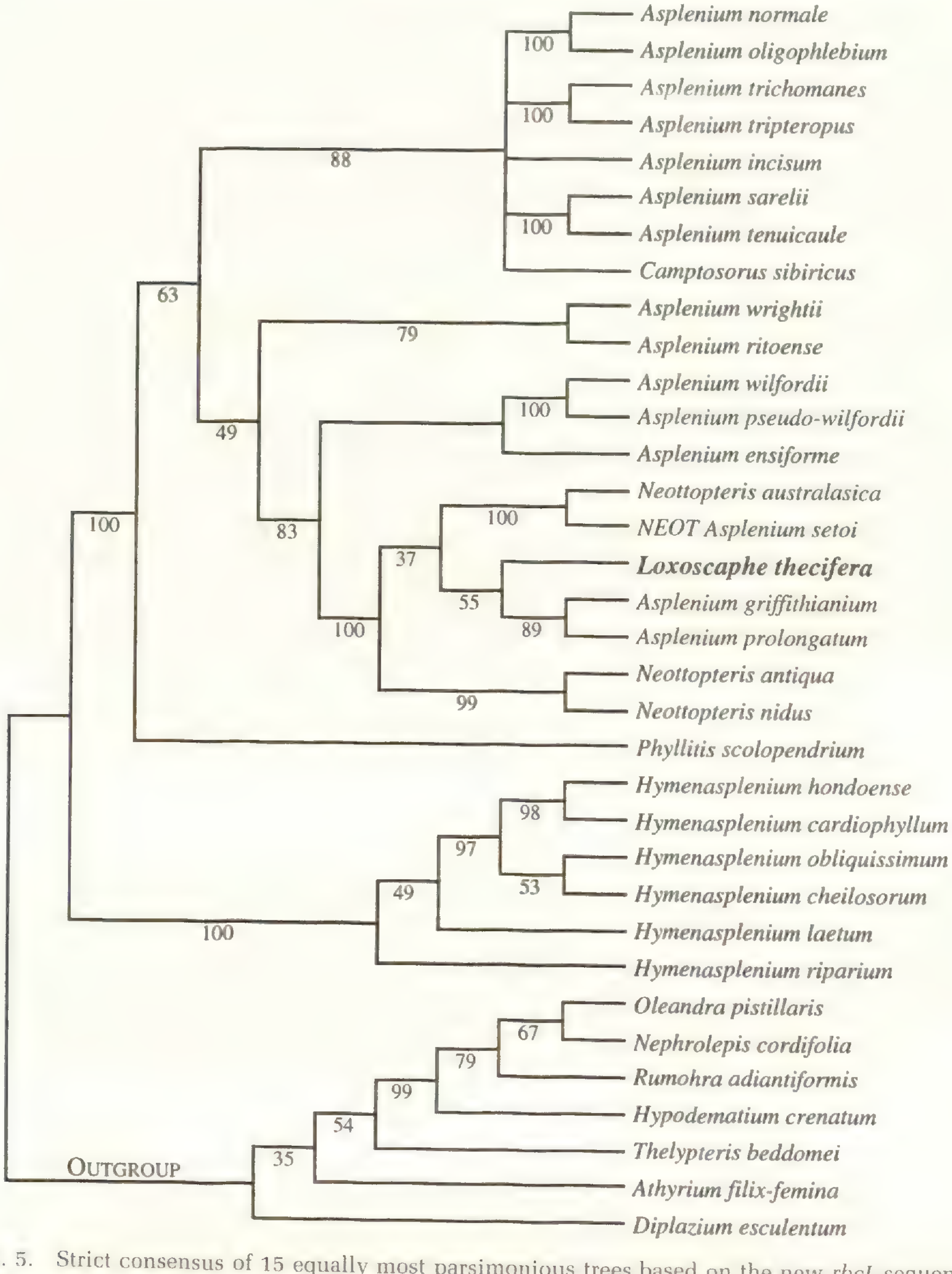

FIG. 5. Strict consensus of 15 equally most parsimonious trees based on the new *rbcL* sequence of *Loxoscaphe thecifera* (bold) plus all *rbcL* sequences of species in Aspleniaceae presently available in GenBank. This tree was generated by a heuristic search with 1000 random addition sequence replicates used to obtain the starting trees. Taxa in the outgroup clade of *Oleandra* through *Diplazium* at the bottom were selected from clades sister to Aspleniaceae in Fig. 4. Bootstrap percentages based on 1000 replicates of five random addition sequence replicates each are provided below the lines. *NEOT* indicates that *Asplenium setoi* (segregated from *Asplenium australasicum* = *Neottopteris australasica* by Murakami et al. [1999a]) would be placed in *Neottopteris* if that genus were accepted and the combination were made.

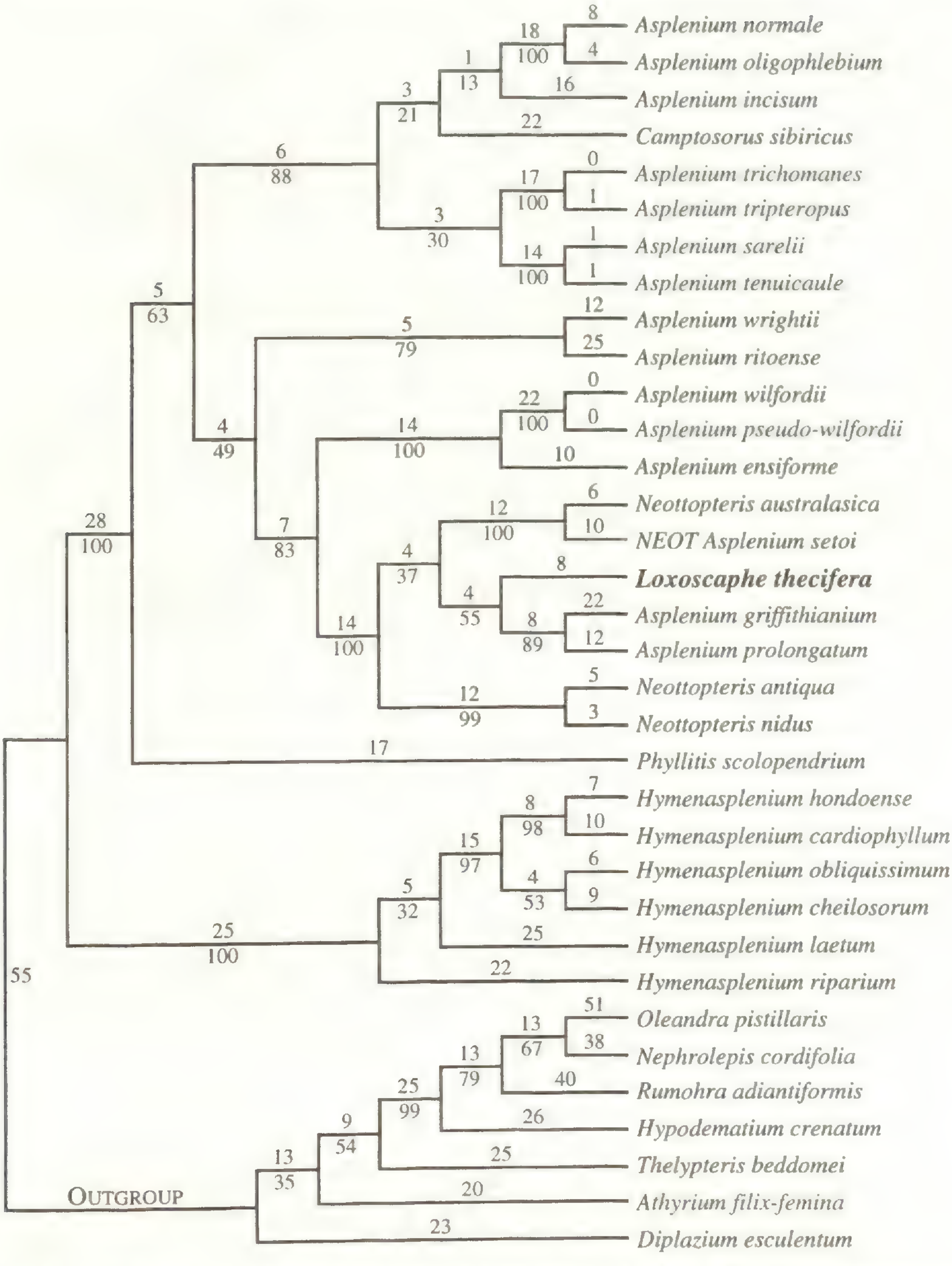

FIG. 6. One of the equally most parsimonious trees randomly selected from 15 represented by the consensus tree of Fig. 5 in order to present branch lengths (synapomorhies and autapomorphies) above the lines and bootstrap percentages as in Fig. 5 below the lines. The position of *Loxoscaphe thecifera* is given in bold. Branch lengths for the subclade *Neottopteris australasica* through *N. nidus*, in which *Loxoscaphe* is nested, are identical for all 15 equally parsimonious trees.

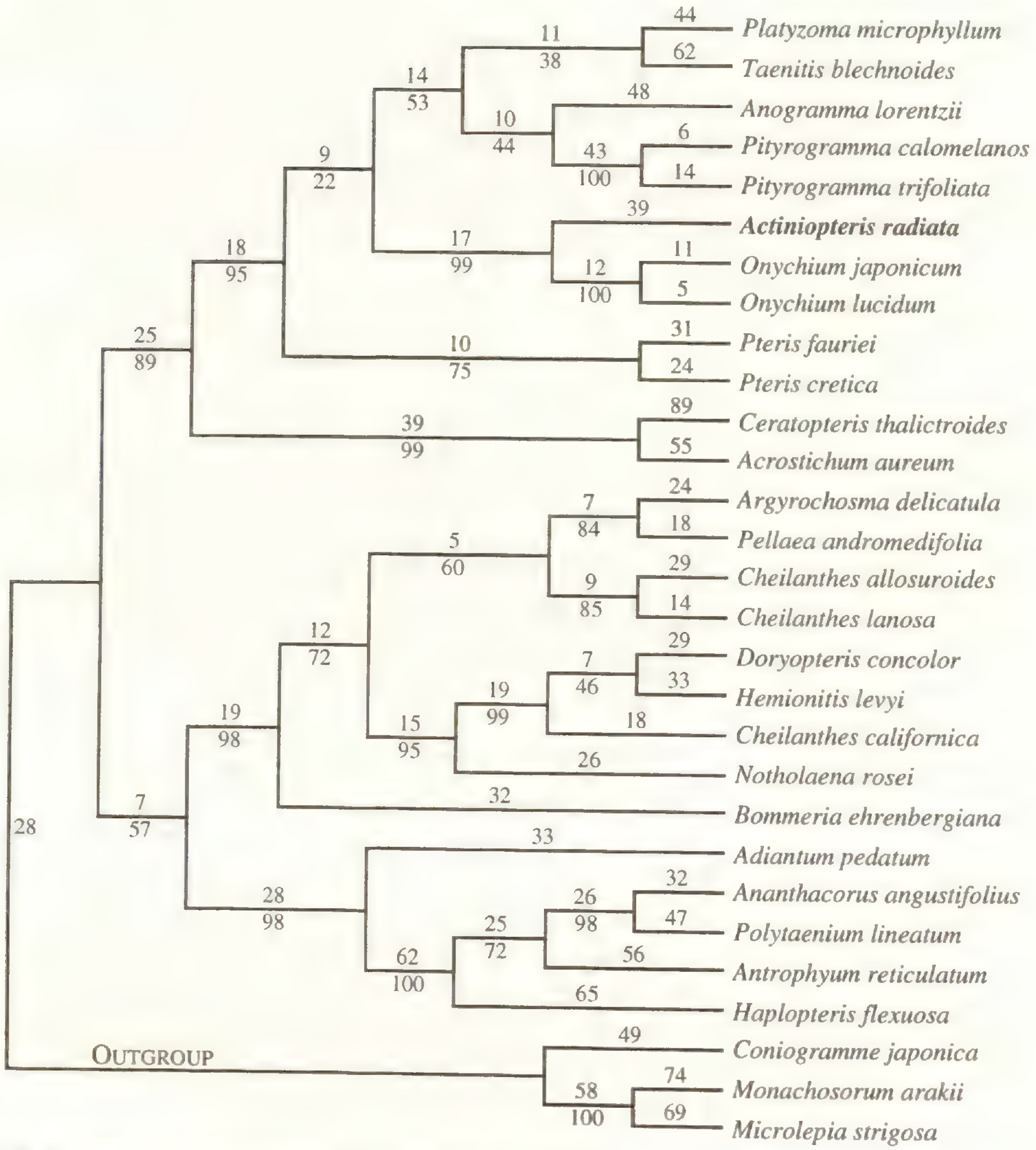

FIG. 7. Single most parsimonious tree based on new *rbcL* sequence of *Actiniopteris radiata* (bold), with other ingroup taxa drawn from the clade of *Platyzoma* through *Vittaria* in Fig. 4 supplemented with sequences of three putatively taenitidoid taxa (two *Pityrogramma* species previously used in Gastony and Rollo [1998] and *Anogramma lorentzii* used here for the first time) and with outgroup taxa *Coniogramme* through *Microlepia* drawn from clades progressively sister to the *Platyzoma* through *Vittaria* clade in Fig. 4. This tree was generated by a heuristic search with 1000 random addition sequence replicates used to obtain the starting trees. Number of synapomorphies supporting each clade and autapomorphic branch lengths for terminal taxa are indicated above the lines. Bootstrap percentages based on 1000 replicates of five random addition sequence replicates each are provided below the lines.

carried out via a maximum parsimony heuristic search of 1000 bootstrap replicates, with the starting tree obtained by stepwise addition based on a random addition sequence of five replicates, the random starting seed number generated by the program, and with TBR branch swapping, MulTrees, and steepest descent in effect. Bootstrapping for the two more focused analyses used the same settings and parameters as the first, except that for the more focused *Actiniopteris* analysis the 1000 bootstrap replicates were based on a random addition sequence of 10 replicates each.

To avoid unnecessary repetition of previously published voucher and GenBank data for species used in our analyses and figures, that information is not presented in Table 1 but can be found as follows (authors of names are given only when not found in the respective following references). See Appendix of Hasebe et al. (1995) for the following species: *Acrostichum aureum*, *Adiantum pedatum*, *Ananthacorus angustifolius*, *Antrophyum reticulatum*, *Arachniodes aristata*, *Argyrochosma delicatula*, *Arthropteris beckleri*, *Asplenium antiquum* (≡ *Neottopteris antiqua* (Makino) Masam.), *Asplenium nidus* (≡ *Neottopteris nidus* (L.) Hook.), *Asplenium ruprechtii* (≡ *Camptosorus sibiricus*), *Athyrium filix-femina*, *Blechnum orientale*, *Blotiella pubescens*, *Bomeria ehrenbergiana*, *Ceratopteris thalictroides*, *Cheilanthes allosuroides*, *Cheilanthes lanosa*, *Coniogramme japonica*, *Cornopteris crenulatoserrulata*, *Ctenitis eatonii*, *Cyathea lepifera* (see *Sphaeropteris lepifera* below), *Cyclosorus opulentus*, *Cystopteris fragilis*, *Davallia denticulata*, *Dennstaedtia punctilobula*, *Deparia bonincola*, *Diplazium esculentum*, *Doodia maxima*, *Doryopteris concolor*, *Dryopteris cristata*, *Elaphoglossum hybridum*, *Gymnocarpium dryopteris*, *Haplopteris flexuosa* (Fée) E. H. Crane as *Vittaria flexuosa* in Hasebe et al. (1995), *Hemionitis levyi*, *Histiopteris incisa*, *Hypodematium crenatum*, *Hypolepis punctata*, *Lindsaea odorata*, *Lonchitis hirsuta*, *Loxogramme grammitoides*, *Loxoma cunninghamii*, *Matteuccia struthiopteris*, *Metaxya rostrata*, *Microlepia strigosa*, *Monachosorum arakii*, *Nephrolepis cordifolia*, *Notholaena rosei*, *Oleandra pistillaris*, *Onoclea sensibilis*, *Onychium japonicum*, *Orthiopteris kingii*, *Paesia scaberula*, *Pellaea andromedifolia*, *Phyllitis scolopendrium*, *Platyzoma microphyllum*, *Polystichum tripteron*, *Polytaenium lineatum*, *Pteridium aquilinum*, *Pteris fauriei*, *Rumohra adiantiformis*, *Sadleria pallida*, *Sphaeropteris lepifera* (Hook.) R. M. Tryon as *Cyathea lepifera* in Hasebe et al. (1995), *Sphaeropteris cooperi*, *Sphenomeris chinensis* (note that the GenBank accession for this uses the synonym *Odontosoria chinensis*), *Stenochlaena palustris*, *Taenitis blechnoides*, *Tectaria gaudichaudii*, *Thelypteris beddomei*, *Vittaria flexuosa* (see *Haplopteris flexuosa* above), *Woodsia polystichoides*. See Table 2 of Murakami et al. (1999a) for the following (when more than one accession of a species is listed in that table, the GenBank accession number is given here to indicate which accession was used in our analysis): *Asplenium australasicum* AB013249 (≡ *Neottopteris australasica* J. Sm.), *Asplenium griffithianum*, *Asplenium setoi* AB013243 (segregated from *A. australasicum* by Murakami et al. [1999a]; would be a species of *Neottopteris* if that genus were accepted; combination apparently not yet made). See Table 1 of Murakami et al. (1999b) for: *Asplenium cardiophyllum* (≡ *Hymenasplenium*

cardiophyllum ≡ *Boniniella ikenoi*), *Asplenium cheilosorum* (≡ *Hymenasplenium cheilosorum*), *Asplenium ensiforme*, *Asplenium hondoense* (≡ *Hymenasplenium hondoense*), *Asplenium incisum*, *Asplenium laetum* (≡ *Hymenasplenium laetum*), *Asplenium normale*, *Asplenium obliquissimum* (≡ *Hymenasplenium obliquissimum*), *Asplenium oligophlebium*, *Asplenium prolongatum*, *Asplenium pseudo-wilfordii*, *Asplenium riparium* (≡ *Hymenasplenium riparium*), *Asplenium ritoense*, *Asplenium sarelii*, *Asplenium tenuicaule*, *Asplenium trichomanes*, *Asplenium tripteropus*, *Asplenium wilfordii*, *Asplenium wrightii*.

Results

The first analysis, designed to place *Loxoscaphe* and *Actiniopteris* within the context of the taxa in Fig. 3 of Hasebe et al. (1995), resulted in a single most parsimonious tree of 3573 steps (Fig. 4) whose general topology is similar to that of Fig. 3 in Hasebe et al. (1995). *Loxoscaphe thecifera* is positioned sister to *Asplenium antiquum* (23 synapomorphies and 100% bootstrap confidence) in the robustly supported (65 synapomorphies, 100% bootstrap value) clade of Aspleniaceae (*Asplenium antiquum* through *Camptosorus sibiricus*) near the center of the tree. This is far removed from genera noted in the literature (Mickel and Beitel, 1988; Copeland, 1947) as having superficially similar soral pouches—*Odontosoria* and its relatives toward the base of the tree (where *Odontosoria chinensis* is reported as *Sphenomeris chinensis*, as it was in Fig. 3 of Hasebe et al. 1995) and *Davallia* near the top. *Actiniopteris radiata* nests deeply within Pteridaceae+Vittariaceae (the clade from *Platyzoma microphyllum* through *Haplopteris flexuosa*) in the lower half of the tree, where its sister relationship to *Onychium japonicum* is strongly supported by 17 synapomorphies and a 97% bootstrap value. *Actiniopteris* is further placed by 22 synapomorphies and a 90% bootstrap value within the clade (*Platyzoma* through *Acrostichum*) that is sister to the clade containing the deeply separated and strongly supported cheilanthoids (*Argyrochosma delicatula* through *Bommeria ehrenbergiana*) that are themselves united by 19 synapomorphies and a 98% bootstrap value.

The more focused analysis designed to position *Loxoscaphe* within a context of all species of Aspleniaceae for which *rbcL* sequences were available in GenBank yielded 15 equally most parsimonious trees of 871 steps whose consensus topology (Fig. 5) is comparable to that in Fig. 1 of Murakami et al. (1999b). Fifteen equally most parsimonious trees were also produced by the similar but not identical data set used by Murakami et al. (1999b), with all variation both there and here attributable to unresolved polytomies in the clade from *Asplenium normale* through *Camptosorus sibiricus* (Fig. 5). The uncertainties of that clade, however, do not affect the positioning of *Loxoscaphe thecifera*, which nests within the fully resolved sister clade from *Asplenium wrightii* through *Neottopteris nidus* in all 15 equally most parsimonious trees. Within that clade, *Loxoscaphe* is placed within the subclade from *Neottopteris australasica* through *N. nidus* by 14 synapomorphies with a boot-

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strap value of 100%, as seen in Fig. 6 of a tree randomly selected from the 15 in order to depict branch lengths. Branch lengths are identical for this *Loxoscaphe* subclade in all of the fifteen equally most parsimonius trees.

The more focused analysis designed to position *Actiniopteris* within an expanded database of taenitidoid and cheilanthoid ferns yielded a single most parsimonious tree of 1611 steps (Fig. 7). The *rbcL* data place *Actiniopteris radiata* sister to the two species of *Onychium* in a clade supported by 17 synapomorphies and a 99% bootstrap value. That robustly supported *Actiniopteris*+*Onychium* clade is in turn sister to one in which the intergeneric relationships are relatively weakly supported (53% bootstrap value), as is the entire clade from *Platyzoma* through *Onychium* (22% bootstrap value). Nevertheless, the *Actiniopteris*+*Onychium* clade is robustly embedded in clades from *Platyzoma* through *Pteris* (95% bootstrap value) and from *Platyzoma* through *Acrostichum* (89% bootstrap value) that are deeply separated from the robustly supported (98% bootstrap) cheilanthoid fern clade (*Argyrochosma delicatula* through *Bommeria ehrenbergiana*).

Discussion

Loxoscaphe.—Results of the taxonomically broad first analysis (Fig. 4) show that *Loxoscaphe thecifera* is robustly nested within Aspleniaceae, providing molecular confirmation of the usual contemporary familial placement of this species group. This permits us to take advantage of the recent *rbcL*-based study of the phylogeny of Aspleniaceae by Murakami et al. (1999b) to provide the broadest available molecular framework within which to assess the relationship of *Loxoscaphe* to species of *Asplenium* sensu lato. The results, presented in Figs. 5 and 6, provide insights useful in resolving the conflicting views of contemporary authors regarding generic segregation of *Loxoscaphe* from *Asplenium* summarized above.

The unresolved clade at the top of Fig. 5 is topologically identical to the unresolved uppermost clade in Fig. 1 of Murakami et al. (1999b) where *Asplenium normale* was represented by three varieties. This lack of resolution may be partly attributable to the greatly truncated sequences available for these taxa in GenBank. PCR products generated for these species by Murakami et al. (1999b) contain bases corresponding only to positions 307–1016 of tobacco *rbcL*, as opposed to PCR products corresponding to positions 1–1381 for *Loxoscaphe thecifera* and other taxa when generated with our primers. *Camptosorus sibiricus* (also known as *Asplenium ruprechtii*) is a member of this unresolved clade, providing no information relevant to its recognition as an independent genus. Whether its morphological similarity to American *Camptosorus rhizophyllus* (*Asplenium rhizophyllum*) indicates true phylogeographic vicariance or is homoplastic remains to be tested by molecular data.

The two most basal ingroups in Figs. 5 and 6 correspond to the segregate genera *Phyllitis* and *Hymenasplenium*, as they did in Fig. 1 of Murakami et al. (1999b). *Hymenasplenium* (= *Asplenium* sect. *Hymenasplenium*) has been intensively studied by Murakami and his colleagues (Mitui et al., 1989; Mu-

rakami, 1992, 1995; Murakami and Moran, 1993; Murakami and Schaal, 1994; Cheng and Murakami, 1998; Murakami et al., 1998a, 1998b, 1999a, 1999b). Because it is distinguishable from *Asplenium* morphologically (Murakami and Moran, 1993) and cytologically (Mitui et al., 1989; but note that *H. costarisorum* shares $x = 36$ with *Asplenium* according to Cheng and Murakami, 1998), the deep basal separation of this clade from the rest of *Asplenium* has been accepted by Murakami et al. (1999b) as warranting recognition of *Hymenasplenium* as an independent genus. Similarly, the deep separation of *Phyllitis scolopendrium*, characterized by paired sori whose indusia open toward each other, led Murakami et al. (1999b) to recommend its recognition as an independent genus needing careful morphological redefinition. They did not provide that morphological redefinition, which would seemingly have to overcome Copeland's (1947, pp. 164–165) argument that the soral generic character of *Phyllitis* would be the same as that of *Diplora*, whose position in the *rbcL* phylogeny of Aspleniaceae is unknown.

The large central clade from *Asplenium wrightii* through *Neottopteris nidus* in Figs. 5 and 6 is only weakly supported by four synapomorphies and a bootstrap value of 49%. The clade from *Neottopteris australasica* through *A. prolongatum*, in which *Loxoscaphe* is embedded, is even more weakly supported (four synapomorphies and 37% bootstrap value), but *Loxoscaphe* is confidently placed within the larger clade from *N. australasica* through *N. nidus* with 14 synapomorphies and a 100% bootstrap value. Of the species in this clade, *N. australasica*, *N. antiqua*, and *N. nidus* were cited by Holttum (1974) as species of *Asplenium* section *Thamnopteris* C. Presl, which is known as *Neottopteris* J. Sm. if given generic rank. Murakami et al. (1999a) differentiated the Japanese specimens of *A. australasicum* (≡ *N. australasica*) sensu Holttum as *A. setoi*; thus the clade from *N. australasica* through *N. nidus* contains four species accepted as members of *Neottopteris* or *Asplenium* section *Thamnopteris*. Murakami et al. (1999b) were surprised by the unexpected sister relationship of *Neottopteris antiqua+nidus* and *Asplenium griffithianum+prolongatum* in their Fig. 1 because *Neottopteris* is defined as having simple leaves in which veins from the midrib run parallel toward the margin, often forking once or less often twice, but with the vein tips always united in a series of short arcs just within the cartilaginous margin of the lamina, the sori being elongate with narrow indusia (Holttum, 1974). *Asplenium prolongatum*, however, has pinnate-pinnatifid leaves that are unique among these four species in having rooting buds at their tips, and *A. griffithianum* has simple leaves whose venation is completely free. Thus *Neottopteris* (*Asplenium* section *Thamnopteris*) as presently defined was paraphyletic in the analysis of Murakami et al. (1999b) and is even more so with *Loxoscaphe* an unexpected and robustly supported member of this clade from *Neottopteris australasica* through *Neottopteris nidus* (Figs. 5, 6).

Although a more comprehensive molecular analysis of the diversity encompassed by *Asplenium* sensu lato is still required, the present study of available *rbcL* data does position *Loxoscaphe* firmly, if enigmatically, within the clade from *Neottopteris australasica* through *N. nidus*. Thus even though eventual

incorporation of *rbcL* data from additional taxa will define clades of other species groups and may modify the relationships of *Loxoscaphe* seen in Figs. 5 and 6, the large number of synapomorphies and 100% bootstrap confidence value with which *Loxoscaphe* is placed within this clade suggest that it will retain its integrity. Moreover, the direct sister relationship of *Loxoscaphe* to *A. griffithianum+prolongatum* based on *rbcL* appears to be supported by morphological characters. Robbin C. Moran (pers. comm.) points out that, based on characters of the lamina, *Loxoscaphe thecifera* belongs to a group of primarily African and Madagascan species informally called the "*Darea* group." Among the other species of this group, he lists *A. griffithianum* and *A. prolongatum*. The monophyletic grouping of these three species by *rbcL* (Figs. 5, 6) supports Moran's view that "*Darea*" is a natural group and suggests that Kramer and Viane (1990, p. 56) may have been misled in regarding "*Darea*" as an artificial group based on superficial resemblance. Finally, based on the evidence in Figs. 5 and 6, *Asplenium* sensu lato would have to be broken into many small and probably ill-defined genera before they and some as yet undefined genus *Asplenium* sensu stricto would not be made paraphyletic by the recognition of *Loxoscaphe* as an independent genus. On the basis of the present study, we therefore conclude that although *Loxoscaphe* is placed with certainty in Aspleniaceaeae (Figs. 4–6), there is presently no phylogenetic justification for its acceptance as an independent genus.

Actiniopteris.—*Actiniopteris* is an Old World genus of five species (Tryon et al., 1990). It is placed in its own family by Pichi Sermolli (1962, 1963) but is more generally treated within Adiantaceae (Crabbe et al., 1975; Kornaś et al., 1982; Jacobsen, 1983) or Pteridaceae (Tryon and Tryon, 1982; Tryon et al., 1990). Tryon and Tryon (1982) divided Pteridaceae directly into tribes and listed *Actiniopteris* and *Onychium* as Old World elements of tribe Cheilantheae (cheilanthoid ferns), not tribe Taenitideae (taenitidoid ferns). The rationale for placing *Actiniopteris* and *Onychium* in the cheilanthoids was not discussed in this book focusing on tropical American ferns, but the authors did note that "The cheilanthoid ferns have been the most contentious group with regard to a practical and natural generic classification" (Tryon and Tryon, 1982, p. 248). In their subsequent taxonomic treatment of Pteridaceae (Tryon et al., 1990), these authors placed *Actiniopteris* and *Onychium* into subfamily Taenitidoideae without explicitly stating their reason for this transfer from the cheilanthoids, but implicating spore morphology in the decision. Their key to subfamilies (1990, pages 231–232) first separates taenitidoids from cheilanthoids on the basis of scales present on the stems in cheilanthoids versus absent in many taenitidoids and secondly on the basis of an equatorial flange present on the spores of taenitidoids that have scales on the stem versus spores without an equatorial flange in cheilanthoids. In this regard these authors were influenced (A. F. Tryon and R. M. Tyryon, pers. comm.) by work already completed for the book on spores of pteridophytes (Tryon and Lugardon, 1991). On page 142 of that work the authors noted that "The arrangement of the flange and ridges in *Onychium* spores is characteristic of the Taenitioid [sic] genera." Tryon and Lugardon (1991) also likened the equa-

torial flange on the spores of *Actiniopteris* to the situation in the taenitidoid genus *Anogramma* and in *Pteris*.

In the parsimony analyses of Hasebe et al. (1995, Fig. 3) the relationships among *Pteris*, *Onychium*, and *Platyzoma+Taenitis* were unresolved as a polytomy. Our analysis used these same *rbcL* sequences plus those of taenitidoids *Anogramma lorentzii*, *Pityrogramma calomelanos*, and *P. trifoliata*, an additional species of *Onychium* (*O. lucidum*), and an additional species of *Pteris* (*P. cretica*). In our analysis (Fig. 7), *Actiniopteris* is robustly grouped with *Onychium* (17 synapomorphies, 99% bootstrap), but the relationships between *Actiniopteris+Onychium* and the other taenitidoid genera and *Platyzoma* are only weakly resolved, with some bootstrap values as low as 22% to 53%. The eventual inclusion of more taenitidoid taxa will probably refine and add confidence to inferred relationships among taenitidoids and between them and *Platyzoma*. At deeper levels in this upper part of Fig. 7, however, the clades from *Platyzoma* through *Pteris* and from *Platyzoma* through *Acrostichum*, within both of which the clade of *Actiniopteris+Onychium* is deeply embedded, are very strongly supported and deeply separated from the well defined clade of cheilanthoids (*Argyrochosma* through *Bommeria*). The present study therefore strongly supports evidence from spore morphology (Tryon and Lugardon, 1991) that *Actiniopteris* and *Onychium* are not cheilanthoids and that the strongest phylogenetic relationships of *Actiniopteris* and *Onychium* are probably with Pteridaceae subfamily Taenitidoideae as hypothesized by Tryon et al. (1990).

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Development of the Sexual Phase of *Pseudocolysis bradeorum* (Polypodiaceae)

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ABSTRACT.—The development and morphology of the sexual phase of *Pseudocolysis bradeorum* (Polypodiaceae) are described from material collected in Finca La Selva, near Puerto Viejo, Province of Heredia, Costa Rica. Spores were sown in Thompson medium with agar (25 Petri dishes) and germinated after seven days; the germination pattern was *Gleichenia*-type and the prothallial development *Drynaria*-type. Gametangia were typical of homosporous leptosporangiate ferns. Sporophytes appeared after seven months of culture. The sexual phase of this species shares many morphological characteristics with Old and New World species of Polypodiaceae. There is a tendency for vegetative propagation with *Pseudocolysis* gametophytes.

Pseudocolysis bradeorum (Rosenst.) L. D. Gómez (Polypodiaceae) is a rare terrestrial fern growing in tropical rain forest soils rich in organic matter, mainly cellulosic. *Pseudocolysis bradeorum* inhabits *Pinus-Quercus-Liquidambar* forests, and coffee and cacao plantations. It has been reported from Mexico (Veracruz, Chiapas), Belize, Nicaragua, Costa Rica, and Panama. Plants have long-creeping rhizomes and sub-dimorphic to dimorphic leaves. Vegetative leaves are simple-lanceolate whereas fertile leaves vary from simple to deeply pinnatifid with long petioles. Venation is anastomosing with veinlets included within the areoles. Sori are regularly to irregularly linear, oblique and exindusiate; the spores are smooth and oblong. The habit of this plant resembles *some Colysis* spp. [= *Leptochilus*, Flora Malesiana, vol. 3, 1998] and *Pleopeltis* (Evans and Mickel, 1969; Gómez, 1977).

Tryon and Tryon (1982) placed Pseudocolysis in the subtribe Polypodieae, but with uncertain relationships. Moran & Riba (1995) suggested a probable hybrid origin because of the highly variable lamina, the variable petiole length, and the occasionally interrupted sori.

Development of the sexual phase of *P. bradeorum* is unknown, although there is information about the morphology of the gametophytes from other genera of Polypodiaceae. For example, Nayar (1962) studied several species of the *Polypodium- Pleopeltis* group [*Arthromeris tenuicaudata* (Hook.) Ching, *A. wallichiana* (Spreng.) Ching, *Campyloneurum angustifolium* (Sw.) Fée, *Colysis elliptica* (Thunb.) Ching, *C. hemionitidea* (Wall.) C. Presl, *C. pedunculata* (Hook. et Grev.) Ching, *Crypsinus griffithianus* (Hook.) Copel., *Crypsinus hastatus* (Thunb.) Copel., *Lemmaphyllum carnosum* (Hook.) C. Presl, *Pleopeltis excavata* (Bory ex Willd.) T. Moore, *Pleopeltis normalis* T. Moore, and *Polypodium amoenum* Wall.].

Prothalli of about 20 genera of Polypodiaceae have been described by several authors (Nayar 1954, 1963a, 1963b, 1964, 1965; Bajpai 1964; Nayar and Chandra 1965; Nayar and Kaur 1969, 1971; Schmelzeisen 1933). Authors reported that, in Polypodiaceae, the development and morphology of the prothalli are perhaps more significant for the understanding of evolutionary processes, in the polypodiaceous ferns than for other fern groups.

Nayar and Raza (1970) studied prothallial development and morphology of three species of Pleopeltidoideae [*Lepisorus loriformis* (Wall.) Ching, *L. thunbergianus* (Kaulf.) Ching and *Weatherbia accedens* (Blume) Copel.], and one species of Polypodioideae (*Polypodium vulgare* L.)]. However, prothallial morphology for most species of Pleopeltidoideae and Polypodioideae is poorly known. Most taxa for which the prothallus has been described belong to subfamilies Microsorioideae, Crypsinioideae, and Platycerioideae.

Several studies are important for our understanding of gametophytes of New World polypods, in particular ones by Stokey (1945, 1954) on *Polypodium pectinatum* L. and *P. plumula* Humb. & Bonpl. ex Willd.; Atkinson & Stokey (1964, 1970) on *Polypodium chnoodes* Spreng.; Reyes and Pérez-García (1994) on *Polypodium lepidotrichum* (Fée) Maxon; Reyes et al. (1996) on *Niphidium crassifolium* (L.) Lellinger; Pérez-García et al. (1998) on three species of *Phlebodium* (R. Brown) J. Smith; and Ramírez and Pérez-García (1998) on *Microgramma nitida* (J. Sm.) A. R. Sm. (see comparative Table 1).

An attempt to classify the various known patterns of spore germination in homosporous ferns was made by Momose (1942), he described three types of spore germination: *centrifugal*, *centripetal*, and *tangential*. Later, Nishida (1965) renamed the *centripetal* and *tangential* types *aspidiod* and *polypodiod*, respectively, and added another which he called the *tripolar* type. Recently, Nayar and Kaur (1968) pointed out that these interpretations of spore germination are incorrect inasmuch as they ignored the polarity of the germinating spore. Nayar and Kaur (1971) gave a detailed account of the patterns of spore germination, classifying them on the basis of the planes of cell division in relation to the polarity of the spore and directions of growth of the primary rhizoid and prothallus. Thus among homosporous ferns there are three distinct categories: *polar*, *equatorial*, and *amorphous*, all differing in the plane and sequence of cell divisions.

The prothallus of the homosporous ferns follows a definite pattern of development leading ultimately to the characteristic adult form. This pattern is constant for each species and commonly to taxa of higher order. In general seven different patterns of prothallial development are recognized among homosporous ferns by Nayar and Kaur (1969):*Adiantum*-type, *Ceratopteris*-type, *Drynaria*-type, *Kaulinia*-type, and *Osmunda*-type. These differ in the sequence of cell divisions, in the stage of development and the region at which a meristem is established, presence or absence of trichomes, and in the final form of the adult thallus.

Thus the type of prothallial development in which an apical cell is established early during the formation of a prothallial plate (*Adiantum*-type) appears to be primitive compared to those in which the establishment of a mer-

TABLE 1. Comparison of the different development stages of the gametophytes of the some genera of the Polypodiaceae family.

Taxa	Type of spore	Types of spore germination	Filamentous phase	Prothallial development
Pseudocolysis bradeorum	Monolete, yellow, ovate to elliptic	Gleichenia-type	Germinal filaments of 2–3 cells long	Drynaria-type
[1]*Arthromeris tenuicauda*	Monolete, brown, plano convex or concavo convex	No mentioned	Germinal filaments of 3–7 cells long	Young prothalli spatulate more or less strap-shaped a cordate
[1]*Arthromeris wallichiana*	Monolete, dark brown, plano convex or concavo convex	No mentioned	Germinal filaments of 3–7 cells long	Young prothalli spatulate more or less strap-shaped a cordate
[1]*Campyloneurum angustifolium*	Monolete, golden yellow, plano convex or concavo convex	No mentioned	Germinal filaments of 3–7 cells long	Young prothalli spatulate
[1]*Colysis elliptica*	Monolete, golden yellow, prominently concavo-convex	No mentioned	Germinal filaments of 3–7 cells long	Young prothalli spatulate more or less strap-shaped a cordate
[1]*Colysis hemionitidea*	Monoloete, golden yellow prominently concavo-convex	No mentioned	Germinal filaments of 3–7 cells long	Young prothalli spatulate more or less strap-shaped a cordate
[1]*Colysis pedunculata*	Monolete, golden yellow, prominently concavo-convex	No mentioned	Germinal filaments of 3–7 cells long	Young prothalli spatulate more or less strap-shaped a cordate
[1]*Crypsinus griffithianus*	Monolete, pale brown, prominently concavo-convex	No mentioned	Germinal filaments of 8–10 cells long	Young prothalli strap-shaped a cordate
[1]*Crypsinus hastatus*	Monolete, pale brown, prominently concavo-convex	No mentioned	Germinal filaments of 8–10 cells long	Young prothalli strap-shaped a cordate
[1]*Lemmaphyllum carnosum*	Monolete, brownish yellow, plano-convex or slightly concavo-convex	No mentioned	Germinal filaments of 3–7 cells long	Young prothalli spatulate
[1]*Pleopeltis excavata*	Monolete, golden yellow, plano-convex or slightly concavo-convex	No mentioned	Germinal filaments of 3–7 cells long	Young prothalli cordate
[1]*Pleopeltis normalis*	Monolete, golden yellow, plano-convex or slightly concavo-convex	No mentioned	Germinal filaments of 3–7 cells long	Young prothalli cordate

TABLE 1. Extended.

Prothallial hairs	Adult prothallus	Sexual expression	Sporophytes
No hairs	Cordiform-spatulate	♂ and ♀, dioic, ♀ type usual, neck 4–6 cells long	7 months
Unicellular papillate with a extracellular cap-like secretion	Cordate with midrib 4–8 cells thick	♂ small, ♀ type usual, neck 4–6 cells long	No sporophyte
Unicellular papillate with a extracellular cap-like secretion	Cordate with midrib 4–8 cells thick	♂ small, ♀ type usual, neck 4–6 cells long	No sporophyte
Unicellular papillate with a extracellular cap-like secretion	Cordate with midrib 4–8 cells thick	♂ small, ♀ type usual, neck 4–6 cells long	No sporophyte
No hairs	Perennial, ribbon-shaped branched	♂ small, ♀ type usual, neck 4–6 cells long	No sporophyte
No hairs	Perennial, ribbon-shaped branched	♂ small, ♀ type usual, neck 4–6 cells long	No sporophyte
No hairs	Perennial, ribbon-shaped branched	♂ small, ♀ type usual, neck 4–6 cells long	No sporophyte
Unicellular papillate with a extracellular cap-like secretion	Cordate with midrib 4–8 cells thick	♂ small, ♀ type usual, neck 5–6 cells long	No sporophyte
Unicellular papillate with a extracellular cap-like secretion	Cordate with midrib 4–8 cells thick	♂ small, ♀ type usual, neck 5–6 cells long	No sporophyte
Unicellular papillate with a extracellular cap-like secretion	Cordate with midrib 4–8 cells thick	♂ globular, ♀ type usual, neck 4–6 cells long	No sporophyte
Unicellular papillate with a extracellular cap-like secretion	Cordate with midrib 4–8 cells thick	♂ small, ♀ type usual, neck 4–6 cells long	No sporophyte
Branched or more complex	Ribbon-shaped	♂ small, ♀ type usual, neck 4–6 cells long	No sporophyte

TABLE 1. Continued.

Taxa	Type of spore	Types of spore germination	Filamentous phase	Prothallial development
[1]*Polypodium ammoenum*	Monolete, hyaline to yellowish, plano-convex or slightly concavo-convex	No mentioned	Germinal filaments of 8–10 cells long	Young prothalli spatulate
[2]*Pseudodrynaria coronans*	Monolete hyaline	No mentioned	Germinal filaments of 3–4 cells long	Young prothalli spatulate
[3]*Lepisorus loriformis*	Monolete, yellowish oil globules	Vittaria-type	Germinal filaments of 4–5 cells long	Kaulinia-type, young prothalli elongate a ribbon-like
[3]*Lepisorus thunbergianus*	Monolete, yellowish oil globules	Vittaria-type	Germinal filaments of 4–5 cells long	Drynaria-type, young prothalli elongate a nearly strap-like
[3]*Weatherbya accedens*	Monolete, yellowish oil globules	Vittaria-type	Germinal filaments of 6–8 cells long	Drynaria type, young prothalli cordate
[3]*Polypodium vulgare*	Monolete, yellowish oil globules	Vittaria-type	Germinal filaments of 6–8 cells long	Drynaria-type, young prothalli cordate
[4]*Polypodium pectinatum*	Monolete	No mentioned	Germinal filaments of 4–8 cells long	No mentioned
[4]*Polypodium plumula*	Monolete	No mentioned	Germinal filaments of 4–8 cells long	No mentioned
[5]*Polypodium chnoodes*	Monolete, brown	No mentioned	Germinal filaments of 3–4 cells long more common, 3–6 cells long	No mentioned, spatulate plate
[6]*Polypodium lepidotrichum*	Monolete, brown	No mentioned	Germinal filaments of 4–5 cells long	No mentioned, spatulate plate
[7]*Niphidium crassifolium*	Monolete, yellow-green	Vittaria-type	Germinal filaments of 4–7 cells long	Drynaria-type, spatulate plate
[8]*Phlebodium araneosum*	Monolete, golden	Vittaria-type	Germinal filaments of 2–7 cells long	Drynaria-type, spatulate plate
[8]*Phlebodium decumanum*	Monolete, golden	Vittaria-type	Germinal filaments of 2–7 cells long	Drynaria-type, spatulate plate
[8]*Phlebodium pseudoaureum*	Monolete, golden	Vittaria-type	Germinal filaments of 2–7 cells long	Drynaria-type, spatulate plate
[9]*Microgramma nitida*	Monolete, yellow	Vittaria-type	Germinal filaments of 4–6 cells long	Drynaria-type, spatulate plate

[1] Nayar, 1962; [2] Nayar, 1954; [3]Nayar & Raza, 1970; [4] Stokey, 1959; [5] Atkinson & Stokey, 1970; [6] Reyes & Pérez-Garcia, 1994; [7] Reyes *et al.*, 1996; [8] Pérez-García *et al.*, 1998; [9] Ramírez & Pérez-García, 1998. ♂ = anteridia, ♀ = archegonia.

TABLE 1. Continued. Extended.

Prothallial hairs	Adult prothallus	Sexual expression	Sporophytes
Unicellular papillate with a extracellular cap-like secretion	Cordate with midrib 4–8 cells thick	♂ cap cell large, basal cell columnar, barrel-shaped, rather than saucer or funnel-shaped	No sporophyte
Unicellular papillate	Cordate	♂ globose and sessile, 2 months ♀ type usual, neck 4–5 cells long, 3–4 month	No sporophyte
Unicellular papillate secretory	Elongate, ribbon-like	Type usual in Polypodiaceae, neck short 3–4 cells	No sporophyte
Unicellular papillate secretory	Elongate cordate	Type usual in Polypodiaceae, neck short 3–4 cells	No sporophyte
Unicellular papillate secretory	Cordate	Type usual in Polypodiaceae, neck short 3–4 cells	No sporophyte
Unicellular papillate secretory	Cordate	Type usual in Polypodiaceae, neck short 3–4 cells	No sporophyte
Unicellular	Cordate	♂ and ♀ type usual, 2–4 months	Sexual
No hairs, only hairs acicular in gametophytes apogamic	Cordate	♂ and ♀ 4 months, type usual	embryo apogamous 3 months
Unicellular papillate in margin, hairs branched in surface	Cordate	♂ globose and elongated, ♀ neck 3–4 cells long	Sexual
Unicellular simple	Cordiform and reniform	♂ globose 3 cells and ♀ usual, dioic	Sexual, 3–4 months
Unicellular papillate	Cordiform with wide wings	♂ globose, ♀ necks 4 cells long	Sexual, 3 months
Unicellular capitate	Cordate-spatulate to cordate-reniform	♂ small, spherical or subglobose, ♀ necks 5–7 cells long	Sexual
Unicellular capitate	Cordate-spatulate to cordate-reniform	♂ small, spherical or subglobose, ♀ necks 5–7 cells long	Sexual
Unicellular capitate	Cordate-spatulate to cordate-reniform	♂ small, spherical or subglobose, ♀ necks 5–7 cells long	Sexual
No hairs	Cordiform-alargate	Monoic, ♂ small barril-shape, ♀ necks 5 cells long	Sexual

istematic cell is delayed (*Drynaria*-type). Usually accompanying this delayed establishment of the meristematic cell there is also a distinct reduction in its activity. Thus in prothalli having an *Adiantum*-type of development, growth and expansion of the young prothallus is mainly by the activity of the meristematic cell. In those having a *Drynaria*-type of development, the meristematic cell does not play a very active part in the growth and expansion of the young prothallus, as is the case in the majority of the Polypodiaceae (Nayar, 1962, 1963a, 1965).

The elimination of the meristematic cell altogether and the establishment of a pluricellular meristem directly from some of the marginal cells of a broad, non-meristic prothallial plate, has been recorded in several cases among the more advanced groups of homosporous ferns. The elimination of meristic cells and the presence of a pluricellular meristem as found in the *Kaulinia*-type of development appears to represent the most advanced condition.

In this paper, we describe the morphology and development of the sexual phase of *Pseudocolysis bradeorum*.

Materials and Methods

Spores of *P. bradeorum* were obtained from fertile specimens of plants (*Gómez 26045*, USJ) growing in Finca La Selva, near Puerto Viejo, province of Heredia, Costa Rica, at an elevation of 50 m. Fertile leaves were sealed in paper envelopes and allowed to dry under natural conditions to favor the release of spores from the sporangia. After several days, the contents of the envelopes were sifted to eliminate sporangial fragments and other debris (Pérez-García *et al.*, 1998).

Spores were sown in 20 Petri dishes (two replicates) on 10% agar supplemented with Thompson medium (Klekowski, 1969; Pérez-García, 1989); one of which was kept in darkness to test for photoblastism. Petri dishes were kept in transparent, sealed plastic bags to avoid dessication and contamination, given a light regime of 12 h light/12 h darkness, with daylight Solar lamps (75w/t 38/A1-D) placed 35 cm above the cultures, and maintained at temperature 25–28°C. Cultures were examined periodically (except the Petri dish maintained in darkness) until germination was detected on the seventh day. After that, dishes were checked every 15 days to record data on the development of the prothallia. Cultures were moistened with sterilized water to prevent desiccation and, in the last stages, to favor the antheridia opening and movement of antherozoids. The culture maintained in darkness was opened 100 days after sowing. Spores were also sown in previously sterilized, natural organic soil.

Photomicrographs were taken from living material with an AO microscope and Tmax B/W film. Pictures of spores were taken with a Zeiss SEM DSM 94DA.

Results

The spores of *P. bradeorum* are monolete, bilateral, yellow, and ovate to elliptic in polar view. They are 56 (54) 53 m long × 34 (33) 32 m broad [n =

50]. The spores are chlorophyllous but also contain yellow oily globules. The thin granulate perine (Figs. 1, 2) is psilate, smooth to verrucate, or variously ornamented. The laesurae are short.

Germination was of the *Gleichenia*-type and commenced seven days after sowing. The first division produced a wall parallel to the polar axis of the spore, cutting off a lateral initial rhizoid cell (Figs. 3, 4). This simple equatorial form is found in many members of the Polypodiaceae.

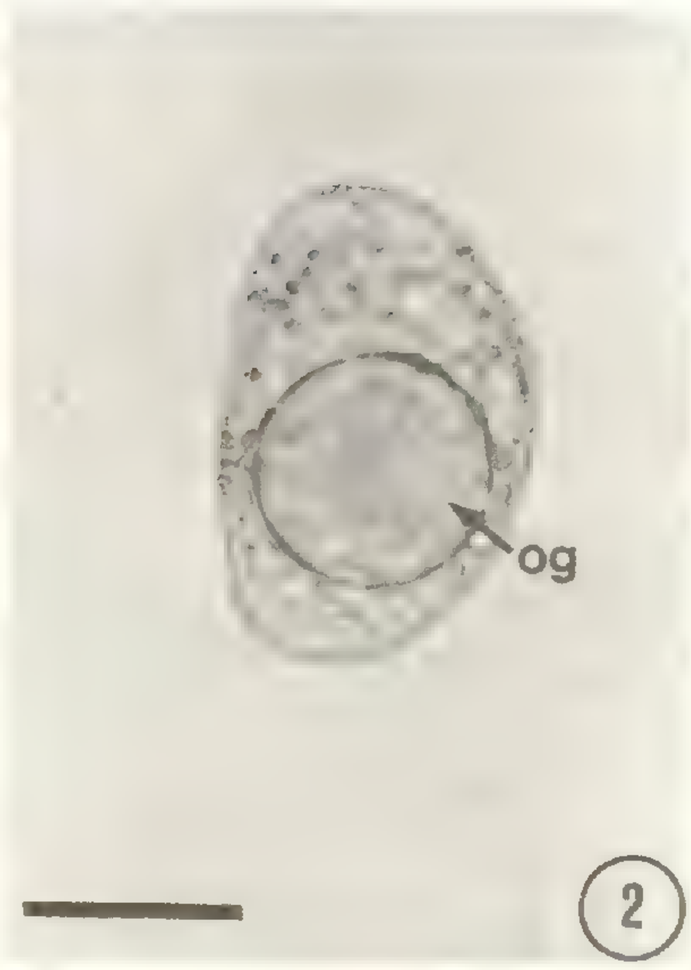
Several transverse divisions of the initial prothallial cell resulted in a uniseriate germ filament 2–3 cells long (each one of these cells with abundant chloroplasts) and a short hyaline rhizoid. The germ filament and the rhizoid cell are oriented in opposite directions (Figs. 3,4). A large oil globule was apparent in the prothallial cells; this is characteristic in many polypodiaceous ferns.

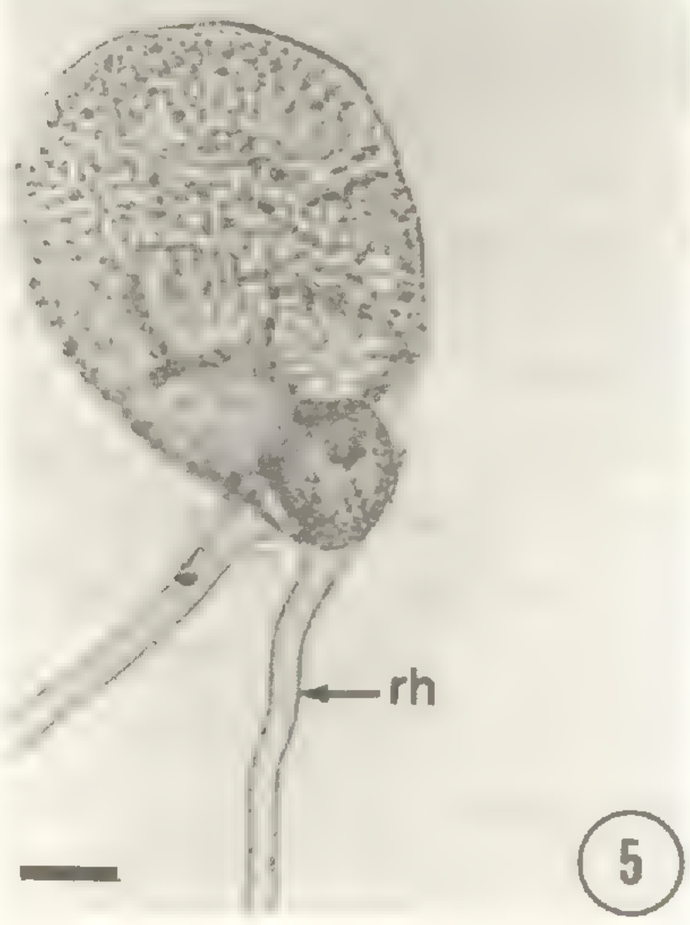
Prothallial development of *Pseudocolysis bradeorum* is of the *Drynaria*-type, in which the differentiation of the apical meristematic cell is delayed, and generally the prothallus develops trichomes along the margin and on both surfaces. Longitudinal and transverse divisions of the filament cells result in a broadly ovate to spatulate prothallial plate at 20–30 days. The plate has two or three hyaline basal rhizoids and 36–50 prothallial cells with abundant chloroplasts. At this time, there is no evident meristematic zone. An obconic meristematic cell is later differentiated as a result of an oblique division of one of the marginal cells from the distal end of the plate. Young prothalli become cordiform-spatulate, and the meristematic cell is replaced by a pluricellular meristem (Figs. 5, 6). At this stage, an inconspicuous cushion is differentiated, the prothalli are glabrous, and the old spore wall is retained on the basal cell. In the *Drynaria*-type development the meristematic cell is not very active in growth and widening of the plate, as it is in most Polypodiaceae (Figs. 7, 8 and 9).

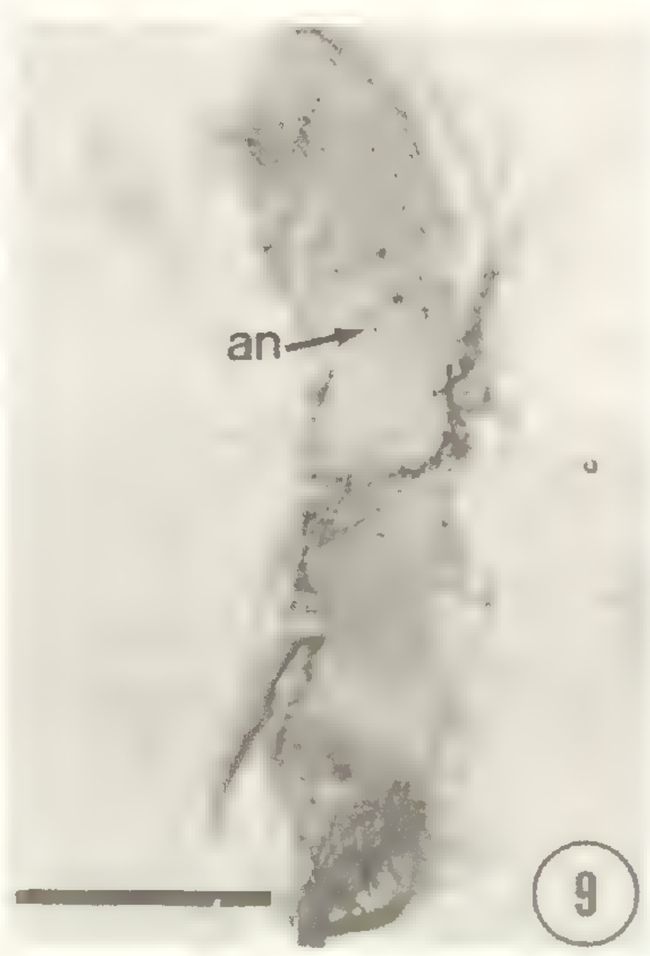
In advanced stages of development, 70–105 days, gametophytes are cordiform-spatulate, with a central shallow meristematic zone, symmetrical wings, abundant short, reddish brown basal rhizoids, and an inconspicuous cushion (Figs. 10, 11).

Antheridia appeared 180 days after sowing; they are globose with three cells, a basal funnel cell, a ring cell, and an opercular cell. In *P. bradeorum*, antheridial dehiscence is accomplished by the detachment of the opercular cell and the opening of a pore in the wall of the androgenic cell (Fig. 12). Archegonia appear on the medial basal part of the cushion 130–140 days after sowing. The archegonial necks are slender and oriented toward the base of the prothallus, with four rows of cells, 5–6 cells per row, plus a binucleate neck cell at maturity (Fig. 13).

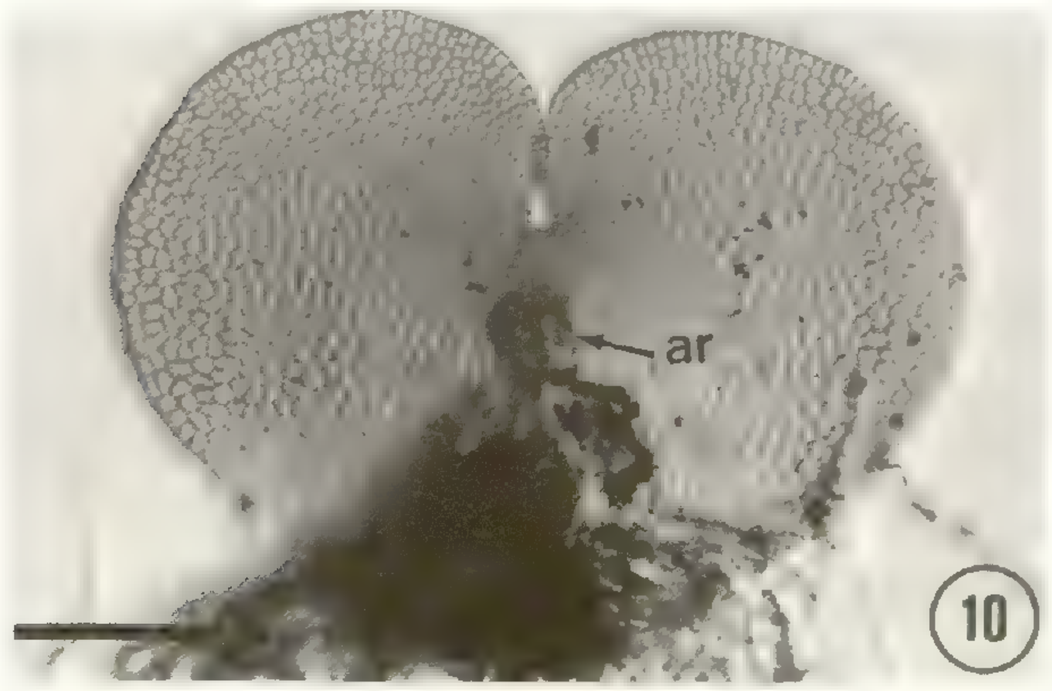
The first leaf of the sporophyte appeared about seven months after spore germination; the petiole is short with small whitish hyaline scales at the base, the lamina is simple and entire, with smooth margins (Fig 14). The venation is open dichotomous. Anomocytic stomata were observed on the abaxial surface (Figs. 15). Sporophytes only developed on the natural organic soil cultures.

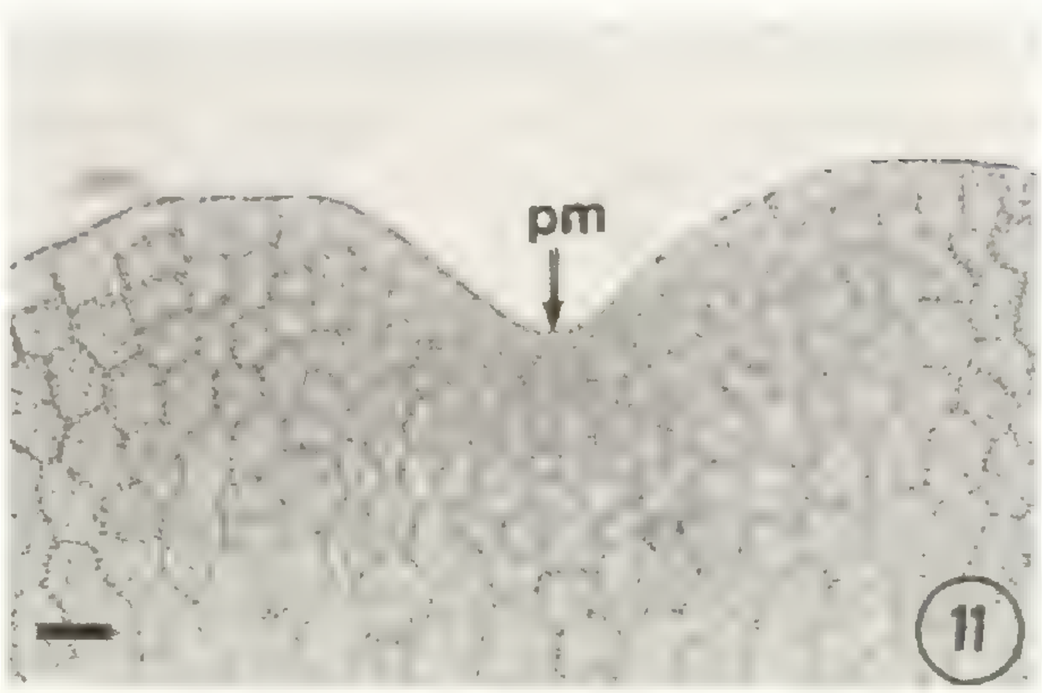


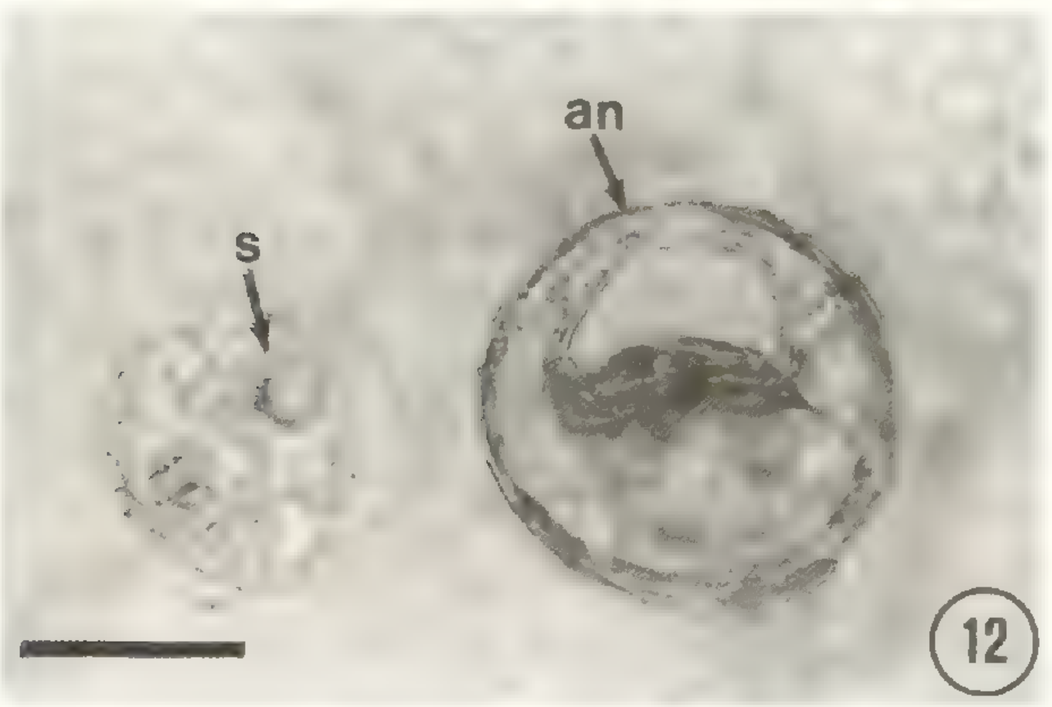


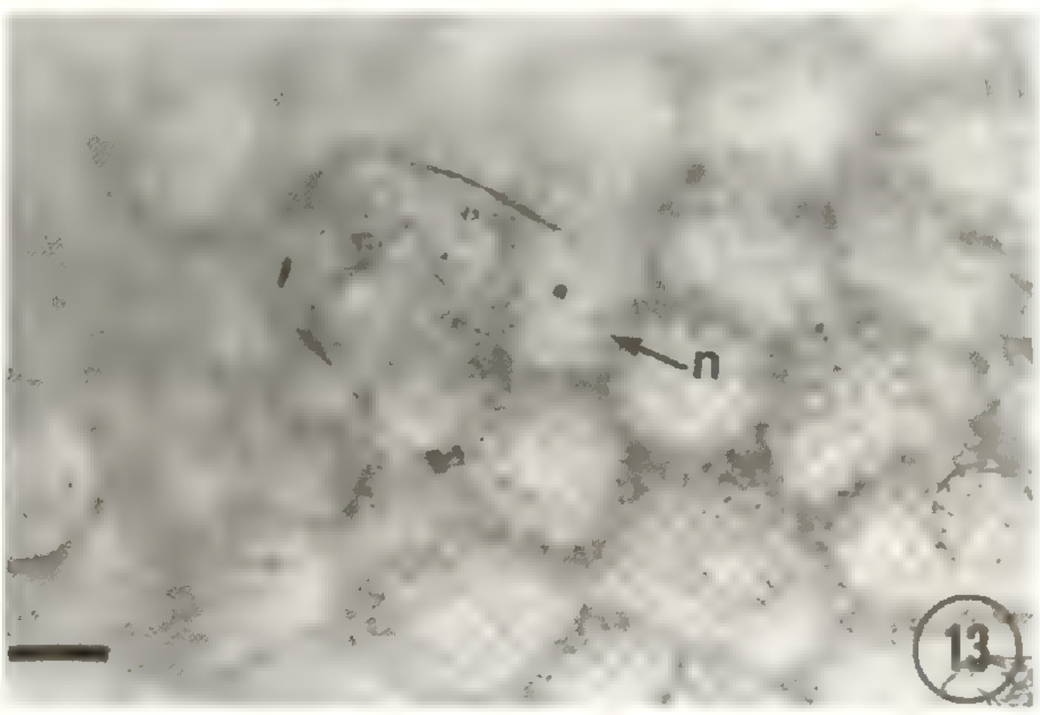


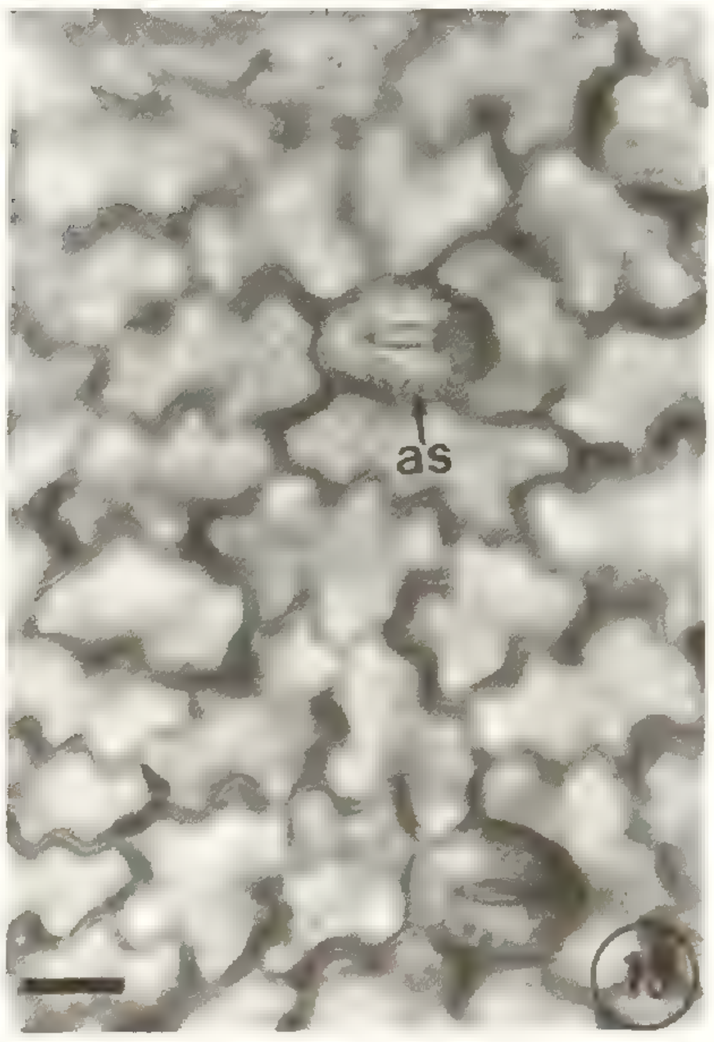
Figs. 1–9. Germination and prothallial development of *Pseudocolysis bradeorum* (Rosenst.) L. D. Gomez. 1. SEM of spore. 2. Spore with yellow oil globule. 3–4. Germination initiation. 5–6. Young prothallial plate. 7–8. Spatulate gametophytes. 9. Spatulate gametophyte with antheridia. Scale bars in figs. 1–6 = 15 μm, in figs. 7–9 = 400 μm.

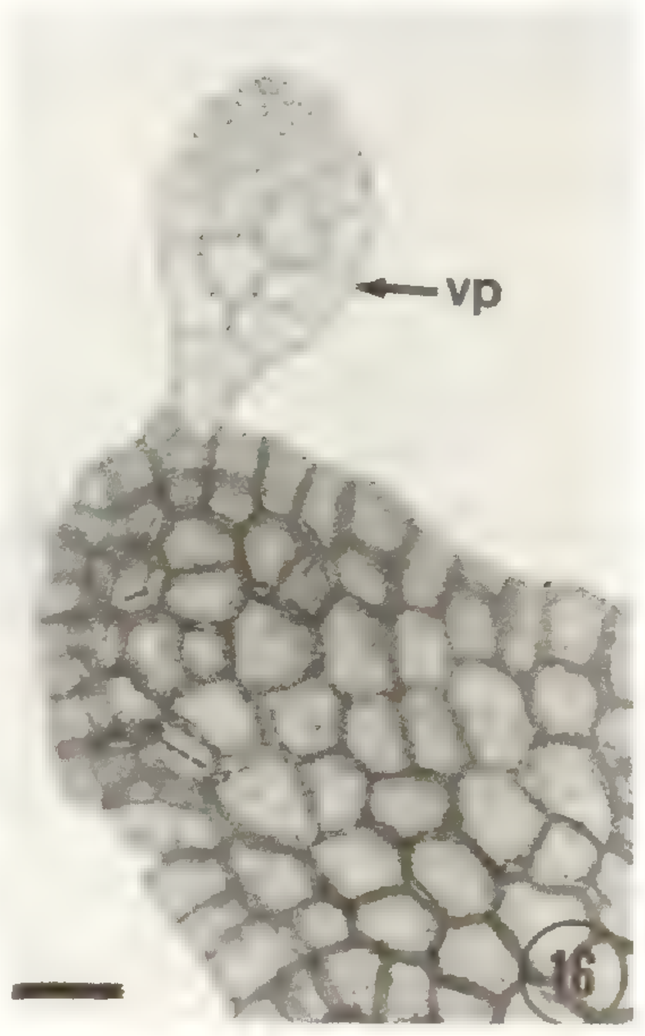












FIGS. 10–16. Morphology of adult prothallus and sex organs of *Pseudocolysis bradeorum* (Rosenst.) L. D. Gómez. 10. Cordate gametophyte with archegonium. 11. Pluricellular meristem. 12. Antheridia. 13. Neck of archegonium. 14. Sporophyte. 15. Anomocytic stomata. 16. Gametophyte showing vegetative propagation. Scale bars in figs. 10, 14 = 400 μm, in figs. 11, 12, 13, 15 and 16 = 15 μm.

After 71 days, we observed the formation of gemmae on the margins of young gametophytes (Fig. 16). When detached, these small gemmae maintained the capacity to grow and developed into adult gametophytes of typical structure and sexuality. The development followed he same pattern observed in agar, except that adult gametophytes were cordate.

Spores kept in darkness did not germinate after 10 days, so this species can be considered to be positively photoblastic.

Discussion

Analysis and comparison of the prothallial development of *Pseudocolysis bradeorum* with other polypodiaceous ferns from the Old and New Worlds, demonstrate that they share many characteristics (see comparative Table 1). However, spores of *P. bradeorum* as well as the spores of *Niphidium crassifolium* (L.) Lellinger, *Microgramma nitida, Phlebodium* spp., and *Polypodium* spp., germinate 7–10 days after sowing, while spores of Old World polypods usually germinate after 30 days (Nayar, 1962).

It is interesting to note that oil globules occur in the first prothallial cell of the germ filament, and the number of cells of the germ filament varies from 2–10. A short filament has been found in several species of Polypodiaceae, e.g., *Polypodium chnoodes* Spr. [*Polypodium dissimile*], *Microgramma nitida* (J. Smith) A.R. Sm. (6 cells), *Polypodium lepidotrichum* (Fée) Maxon (4–5 cells), *Niphidium crassifolium* (3–7 cells), *Phlebodium araneosum, P. pseudoaureum,* and *P. decumanum* (4–7 cells), *Pleopeltis excavata* (6 cells), *Crypsinus* (9 cells), *Arthromeris* (6–7 cells) and *Pseudodrynaria coronans Ching* (3, 7,10 cells).

The *Gleichenia*-type germination pattern shown by *Pseudocolysis bradeorum* is also common in advanced groups of homosporic ferns, such as Polypodiaceae. In this pattern, the germ filament and primary rhizoid elongate in opposite directions along the equatorial plane of the spore. This may be a derived character.

Two types of germination patterns occur in the Polypodiaceae, the *Vittaria*-type and the *Gleichenia*-type. Basal groups such as Platycerioideae exhibt the *Gleichenia*-type, and comparatively derived groups as the Crypsinoideae, Drynarioideae, and Microsorioideae have the *Vittaria*-type. In this regard, the germination type of *Pseudocolysis bradeorum* is "primitive".

In general, the temporary or permanent suppression of organized apical growth appears to be the key note in the evolution of the prothalli of homosporous ferns. Prothallial development is *Drynaria*-type, in which differentiation of the meristematic cell is delayed longer than in the *Adiantum*-type. In Polypodiaceae, prothallial development in basal genera of the Microsorioideae (Nayar, 1962; 1963a and 1963b) and Platycerioideae (Nayar and Chandra, 1965) is closer to the *Adiantum*- type, while in the more derived groups as the Drynarioideae (Nayar, 1965) and Crypsinoideae (Nayar, 1962), the development is *Drynaria*-type, with delay of the formation of the meristematic cell. In comparatively more advanced genera such as *Kaulinia* (Nayar, 1963a), *Colysis* and *Paraleptochilus* (Nayar, 1963b), and *Colysis* (Nayar, 1962) the *Kaulinia*-type prevails.

Some Polypodiaceae (Microsorioideae and Pleopeltoideae) have a prothallial development of the *Kaulinia*-type, with no differentiation of a meristematic

cell, the prothalli is prolonged and ribbon-shaped. This condition can be considered as an advanced condition (Atkinson, 1973).

In regards to the shape of the adult gametophytes, a wide variation can be found, long-cordate in *Niphidium* and *Polypodium* spp., cordate to reniform, spatulate and long-lived in *Polypodium* spp. and *Crypsinus*, strap-shaped in *Colysis* and *Pleopeltis*. In *Pseudocolysis* the prothallia are cordate-spatulate to strap-shaped.

Gametangia are of the common type for homosporous leptosporangiate ferns. Shape and structure of the gametangia agree with the description of those organs given by Davie (1951), Hartman (1931), and Nayar (1962) for some polypodiaceous ferns. This is characteristic of polypodiaceous ferns and previously described in classical papers by Schlumberger (1911) and Hartman (1931).

Stokey (1959) mentioned the presence of unicellular papillate trichomes with an extracellular cap-like excretion, on margins and both surfaces of the prothalli. This is a common condition found in many polypodiaceous ferns (*Campyloneurum, Microgramma, Niphidium, Phlebodium, Pleopeltis,* and *Polypodium*). However, the gametophytes of *Pseudocolysis* are glabrous, a condition which, according to Nayar (1962), is advanced.

The first leaf of the sporophyte appeared seven months after spore germination. There is wide variation reported in the age of sporophyte differentiation. Species of *Niphidium* and *Polypodium* appeared after three months, while in *Phlebodium* spp., time until sporophyte production is between five months and 2.5 years (Pérez-García *et al.*, 1998).

Vegetative propagation may allow gametophytes to survive extreme environmental conditions and to propagate vegetatively thus permitting survival of the species in more extreme ecological niches.

Results obtained in this study, in regard to development and characteristics of the gametophytes and comparison with data from New and Old World genera of polypodiaceous ferns, confirm that *Pseudocolysis bradeorum* is closely related to some genera of Polypodiaceae.

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American Fern Journal 91(4):227–229 (2001)

Shorter Notes

New Records of Pteridophytes in the Semi-Arid Region of Brazil.—In Brazil, 70% of the Northeast region, including the northern portion of the state of Minas Gerais, is covered by semi-arid vegetation. The state of Pernambuco, for example, has a total area of 98,079 km^2, of which 60,000 km^2 is covered by typical caatinga vegetation (Egler, Rev. Bras. Geog.13: 65–78. 1951; Sampaio, pp. 35–63 *in* Bullock and Davidse, *Seasonally Dry Tropical Forest*, 1995).

Since water deficiency appears to be the key factor in limiting the occurrence of pteridophytes in seasonally dry tropical areas, there are relatively few species of pteridophytes known from semi-arid regions or caatinga vegetation. Nevertheless, a few species of pteridophytes are well adapted to survival in such areas (Kornás, pp. 391–395 *in* Dyer and Page, *Biology of Pteridophytes*, 1985). For Peru, Tryon (Cont. Gray Herb. 194:1–253. 1964), records 176 native species of pteridophytes across four vegetation types, including the xeric Sierra Steppe and Scrub vegetation. Also many members of the Pteridaceae, Tribo Cheilantheae, have been found in the xeric and semixeric regions of central and northeastern Mexico (Tryon & Tryon, 1982, *Ferns and allied plants, with special reference to tropical America*). Other works refer to the occurrence of pteridophytes in the dry environments of Africa (Kornás, Acta Soc. Poloniae 46:669–690. 1977), and in Australia the genus *Cheilanthes* is noted for its ability to survive in low humidity conditions (Quirk & Chambers, Fern Gaz. 12:121–127. 1981). In Brazil, Barros *et al.* (Biol. Bras.1: 143–159. 1989) have documented nine species of pteridophytes in the caatinga vegetation of Pernambuco.

The goal of this paper is to enumerate the pteridophytes that occur in the semi-arid region of the State of Pernambuco, Brazil, document three new records, and add information about the habitat preferences of these plants.

Pteridophytes were collected in Petrolina (90°09'S, 40°22'W), which is situated in the semi-arid region of Pernambuco, northeastern Brazil. This area is located at an altitude of 365 m in a region known as the Sertaneja Depression. The climate is characterized by extended dry periods alternating with ephemeral rains. The region corresponds to type BSwh, of Köppen's system (Embrapa, 1993. *Zoneamento Agroecológico do Nordeste*) and the annual rainfall is 510 mm. The vegetation is xerophytic, with Bromeliaceae and Cactaceae being especially representative (Egler, Rev. Bras. Geog.13:65–78. 1951)

Species identifications were carried out using Alston *et al.* (Bull. Brit. Mus. Nat Hist. 9:233–330. 1981), Proctor (Bull. Brit. Mus. Nat Hist. 7:3–5. 1985), Mickel & Beitel (Mem. New York Bot. Garden 46:1–568. 1988), and Tryon & Stolze (Fieldiana Bot. 29:1–80. 1992). Voucher specimens are deposited in TSAH, the herbarium of the Tropical Semi-Arid Agricultural Research Center—Embrapa (Brazil).

Acrostichum danaeifolium Langsd. & Fisch. [*Ambrósio 55,* TSAH 1728

&1729], *Thelypteris interrupta* (Willd.) Iwatsuki [*Ambrósio s.n.*, TSAH 1721], and *Marsilea quadrifolia* L. [*Ambrósio 52,* TSAH 1723] are recorded for the first time in the Brazilian semi-arid region. *Thelypteris interrupta* and *A. danaeifolium* were found to be common in humid soils of the caatinga, near irrigation channels. These species probably colonized the region naturally with the advent of modified habitats as niches became available for their survival. Tryon (Cont. Gray Herb.194:1–253.1964) observed that, in Peru, non-native species have become naturalized along irrigation channels or in areas that become more mesic as a result of their presence. *Marsilea quadrifolia* occurs in aquatic environments, such as rivers and streams and, according to Allsopp (Nature 169:79–80.1952), members of the genus are probably able to survive drought conditions due to their desiccation resistant sporocarps. Kornás (*in* Dyer and Page, *Biology of Pteridophytes,* 1985) also comments upon the occurrence of pteridophytes in areas where the climate is extremely dry, as in deserts or sub-desert regions of tropical Africa. *Thelypteris* and *Marsilea* are among the genera that have been found there.

Anemia tomentosa (Sav.) Sw. var. *tomentosa* [Ambrósio *54,* TSAH 1724], *Pityrogramma calomelanos* (L.) Link. var. *calomelanos* [*Ambrósio 51,* TSAH 1725], *Selaginella convoluta* (Arn.) Spring [*Ambrósio 53,* TSAH 1722], *S. sellowi* Hieron. [*Ambrósio s.n.*, TSAH 1726], and *Azolla microphylla* Kaulf [*Ambrósio 61,* TSAH 1727 & 1730] were also collected from Petrolina. These species have previously been reported from other semi-arid regions of Brazil (Barros *et al.*, Biol. Bras. 1:143–159.1989). In their floristic survey, Barros *et al.* also reported *Anemia villosa* Humb. & Bonpl. Ex Willd., *Anemia oblongifolia* (Cav.) Sw., *Hemionitis tomentosa* (Lam.) Raddi., *Polypodium polypodioides* (L.) Watt., and *P. hirsutissimum* Raddi. as part of the vegetation of the caatinga. The most notable pteridophyte of the semi-arid region is certainly *S. convoluta,* the poikilohydrous type, also observed by Morelo (Rev. Bras. Biol. 14:83–108. 1954). This reviviscent species has physiological characters that confer resistance to hydric stress; they curl up and remain alive through the drought, unfolding each time they are moistened (Rawistscher *et al.*, An. Acad. Bras. Ciên. 21: 287–301. 1952). Although pteridophytes are uncommon in xeric environments, especially when compared to areas with more mesic edafoclimatic conditions (Ambrósio and Barros, Acta Bot. Bras. 11:105–113. 1997), they are becoming increasingly documented and studied (Kornás, Acta Soc. Poloniae 46:669–690. 1977, and other references above). The three new records discussed above are intended to show that the distribution of pteridophytes in xeric regions remains still largely undocumented.

The authors are grateful to the Tropical Semi-Arid Agricultural Research Center—Embrapa / Petrolina—PE for the use of its premises and equipment, Dr. Alexandre Salino, Federal University of Minas Gerais, for his suggestions, Dra Leonor Costa Maia, Federal University of Pernambuco, for reviewing the manuscript, and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for financial support.— SANDRA T. AMBRÓSIO, Center for Biological Sciences, Federal University of Pernambuco, Recife—PE, and NATONIEL F. DE MELO, Center for Biological Sciences, Federal University of Pernambuco,

Recife—PE and Tropical Semi-Arid Agricultural Research Center—Embrapa, Petrolina—PE.

The Typification, Identity, and Distribution of *Cyathea mamillata* Fée.–The publication of *C. mamillata* by Fée (Crypt. Vasc. Brés. 1:176, t. 63, f. 2. 1869) is based on a specimen attributed to Galeotti: "Habitat in Serra-Carassa Brasiliae (*Galeotti, no. 247*). [H.F.]." Morton (Amer. Fern J. 61: 62–63. 1971) pointed out that Galeotti collected ca. 8,000 numbers of herbarium specimens in five years, mostly in Mexico, plus a few in Cuba, and that, unlike most early collectors, he collected many duplicates and sold them widely. Although Galeotti is often cited as the collector of plants from Brazil, Venezuela, and Colombia, in fact, he never travelled in South America; he was merely the distributor of the plants attributed to him from there. Wurdack (Taxon 19:911–913. 1970) has noted false data provided by Galeotti on specimens he distributed from Brazil. See also Glaziou (Soc. Bot. France Mém. 3:1–7. 1905) for his own account of the Brazilian collections that he attributed to himself.

After 1853, when Galeotti was appointed as Director of the Jardin Botanique de Bruxelles, many specimens collected by Linden, Funck, Schlim, Ghiesbreght, Lindig, and Claussen were mislabelled and distributed by Galeotti. We have found that the label on the type specimen cited by Fée (P, not seen) is in error as to the identity of the collector. An isotype of *C. mamillata* is plainly labelled *Claussen 247* (BR). Probably, the label of *C. mammilata* was changed by Galeotti when a specimen was sent to Paris.

Tryon, in a paper about the American tree ferns allied to *Sphaeropteris horrida* (Rhodora 73: 17. 1971), synonymized *C. mamillata* Fée as a synonym of *S. gardneri* (Hook.) R. M. Tryon, but he did not correct the name of the collector. We confirm Tryon's identification, based on our examination of the isotype. This species is endemic to southern Brazil and can be found in the states of Minas Gerais, Rio de Janeiro, São Paulo, and Santa Catarina. It occurs from 250 to 1300 m elevation, along rivers and in shaded places. The main features to recognize *S. gardneri* are: small leaves (ca. 1 m long), concolorous paleaceous scales on the stipe base, simple or branched hairs on the costules abaxially, and rarely a few flattish scales on abaxial surface of the segments.

In addition to the isotype of *C. mamillata,* we examined the following specimens from southeastern Brazil: **Minas Gerais**: Barbacena, *Glaziou 11710* (BR, NY, P not seen); same locality, *Pohl s.n.* (BR, P not seen): Ouro Fino, *Hoehne s.n.* (SP 19594); Ouro Preto, *Macedo 2885* (SP); Vila Rica, *Pohl 3767* (BR); Caldas, *Mosén 2033* (BR, P not seen). **Rio de Janeiro**: Petrópolis, *Spannagel 434* (NY); Nova Friburgo, *Glaziou 7329* (NY, P not seen); Tijuca, *Glaziou 1707* (BR). **São Paulo**: Santana, *Brade 5378* (NY); Osasco, *Luederwaldt s.n.*, ex. Herb. Museu Paulista no. 6624 (SP 634).

We are grateful to David B. Lellinger for detailed and constructive comments on the manuscript.—JEFFERSON PRADO and PAULO H. LABIAK, Instituto de Botânica, Seção de Briologia e Pteridologia, C. P. 4005, 01061-970 São Paulo—SP, Brazil.

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Review

Flora of Florida, Volume I, Pteridophytes and Gymnosperms, by Richard P. Wunderlin and Bruce F. Hansen. 2000. University Press of Florida, 15 NW 15th St, Gainesville, FL 32611–2079. xii, 365 pp. Hardcover (ISBN 0-8130-1805-6) $49.95.

Of late, the state of Florida is awash with new pteridophytic literature. Beginning in 1998 with Richard Wunderlin's "Guide to the Vacular Plants of Florida," continuing earlier in 2000 with Gil Nelson's "The Ferns of Florida," and most recently with this fine new volume, enthusiasts of the rich Florida fern flora have perhaps more information than they can handle. The present volume is an update and expansion of Wunderlin's earlier work, which is a condensed manual consisting mainly of a set of keys to permit determination of the more than 3,800 species of vascular plants known to occur in Florida, with little supplementary information. The more detailed encyclopedic treatment of the flora will require an anticipated eight total volumes.

Pteridophytes occupy only part of the first volume. The first 99 pages are introductory material, sure to be of interest to all readers. These chapters contain background information on Florida's physical features and vegetation, a fascinating botanical history of the state, and notes on using the book. The 18 species of gymnosperms take up only 27 pages of text. The remainder of the rest of the volume includes a summary of the families, genera, and species treated, as well as bibliographies and indices.

The pteridophyte portion is based in part on the work of the late Cliff Nauman, who is listed (followed by Wunderlin and Hansen) as first author of the treatment and to whom the volume is dedicated. The 162 species of ferns and fern allies are grouped into 60 genera in 23 families. The familial classification follows that of Kramer and Tryon in the "Families and Genera of Vascular Plants" volume, which is slightly different from those both in the "Flora of North America" volume and in Wunderlin's earlier manual. The keys to families, genera, and species are indented and seem to work quite well. Each taxon has a description tailored to Florida examples; so, for example, the generic descrition of *Huperzia* reads "plants epiphytic" because the sole Florida species (*H. dichotoma*) is an epiphyte, even though other U.S. species are terrestrial. Following each species description is a statement of habitats and distribution (both in Florida and in general), often followed by discussions of taxonomic problems and related species. These discussions add greatly to the volume, although a few may seem confusing to beginners, such as the treatment of *Lycopodiella caroliniana,* which discusses how, "Diploid ($2n = 70$) and tetraploid ($2n = 140$) plants produce sterile triploids ($3n = 105$) when they cross."

Sixty species are illustrated by line drawings, most singly on full-page plates drawn by Priscilla Fawcett and Wes Jurgens. Some of Fawcett's drawings are

reproduced from Correll and Correll's "Flora of the Bahamian Archipelago" but most are new. A few of the plates could have been reproduced with a bit less contrast, but all of them are informative, detailed, beautifully composed works of art.

The question arises whether Florida fern fanciers should buy copies of both the Flora volume and Gil Nelson's "The Ferns of Florida" (reviewed recently by John Mickel in Fiddlehead Forum 27[5]:41, 2000). To me, the two books complement one another nicely. Like most field guides, Nelson's alphabetically arranged book lacks keys, but it is more fully illustrated than the Flora volume, including 204 color photographs, 32 figures, and small maps. The field guide also contains checklists and a glossary, as well as more conservation-related information. Because Nelson and the "Flora of Florida" authors apparently worked in fairly close correspondence, the two books are easily cross-referenced, although minor differences in taxonomic interpretations account for slight diiscrepancies in overall numbers of accepted species and genera. "Flora of Florida" is more detailed in its introductory chapters and provides better means of distinguishing species in taxonomically complex genera like *Asplenium, Salvinia,* and *Thelypteris,* and also provides more information on taxonomy and classification. The drawings in the Flora volume also generally are more detailed than those in Nelson's guide, in spite of less than half of the species being illustrated. It should be noted that the Flora is supported by an atlas of plant distributions, available on the Web (http://www.plantatlas.usf.edu) and on CD-ROM.

Typographically, the Flora volume is relatively user-friendly, with the text nicely laid out in an easy to read single-column format. Readers who stumble upon the unusual "*Campyloneurum angu*" on p. 272 should note that the rest of the species epithet (*stifolium*) was accidentally transposed to the righthand margin of the same line. Otherwise, a quick perusal turned up no major problems.

In summary, Wunderlin and Hansen's inaugural volume of the "Flora of Florida" sets a very high standard for the rest of the series. The pteridophyte treatment is authoritative, accurate, and detailed, and will surely satisfy the inquiring minds of Florida pteridophiles, both professional and amateur. I congratulate the authors on a fine accomplishment and await the rest of the series eagerly.—GEORGE YATSKIEVYCH, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299.

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Referees for 2001

All papers submitted to the journal are peer reviewed. Members of the editorial board and the Society, as well as additional scientists in cognate areas, do these reviews on a voluntary basis. It is their work that contributes to the high quality of articles in the American Fern Journal and to its continued success. The American Fern Society and I extend our thanks to the following reviewers for their assistance, diligence, and patience in the year 2001—R. James Hickey.

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